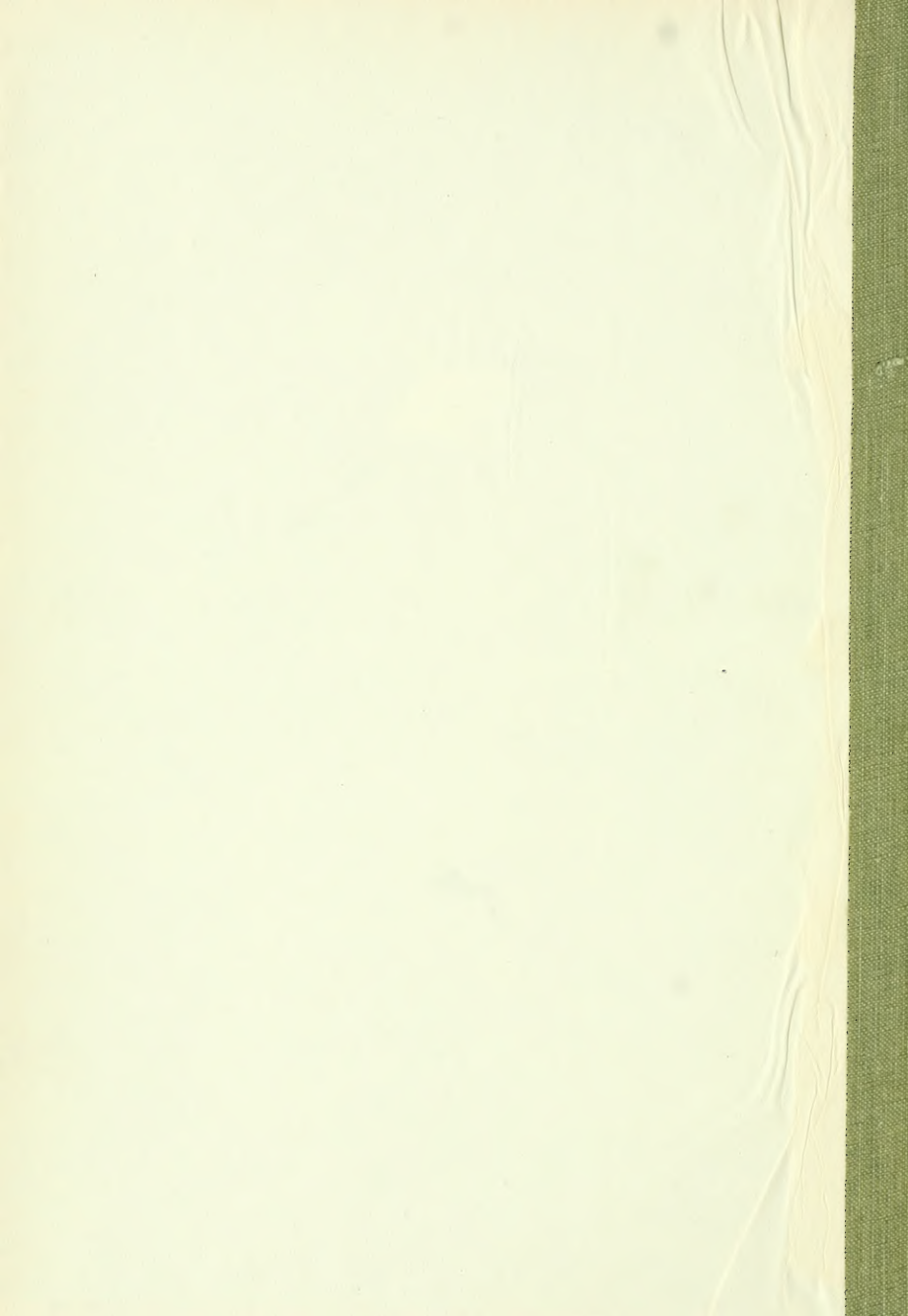
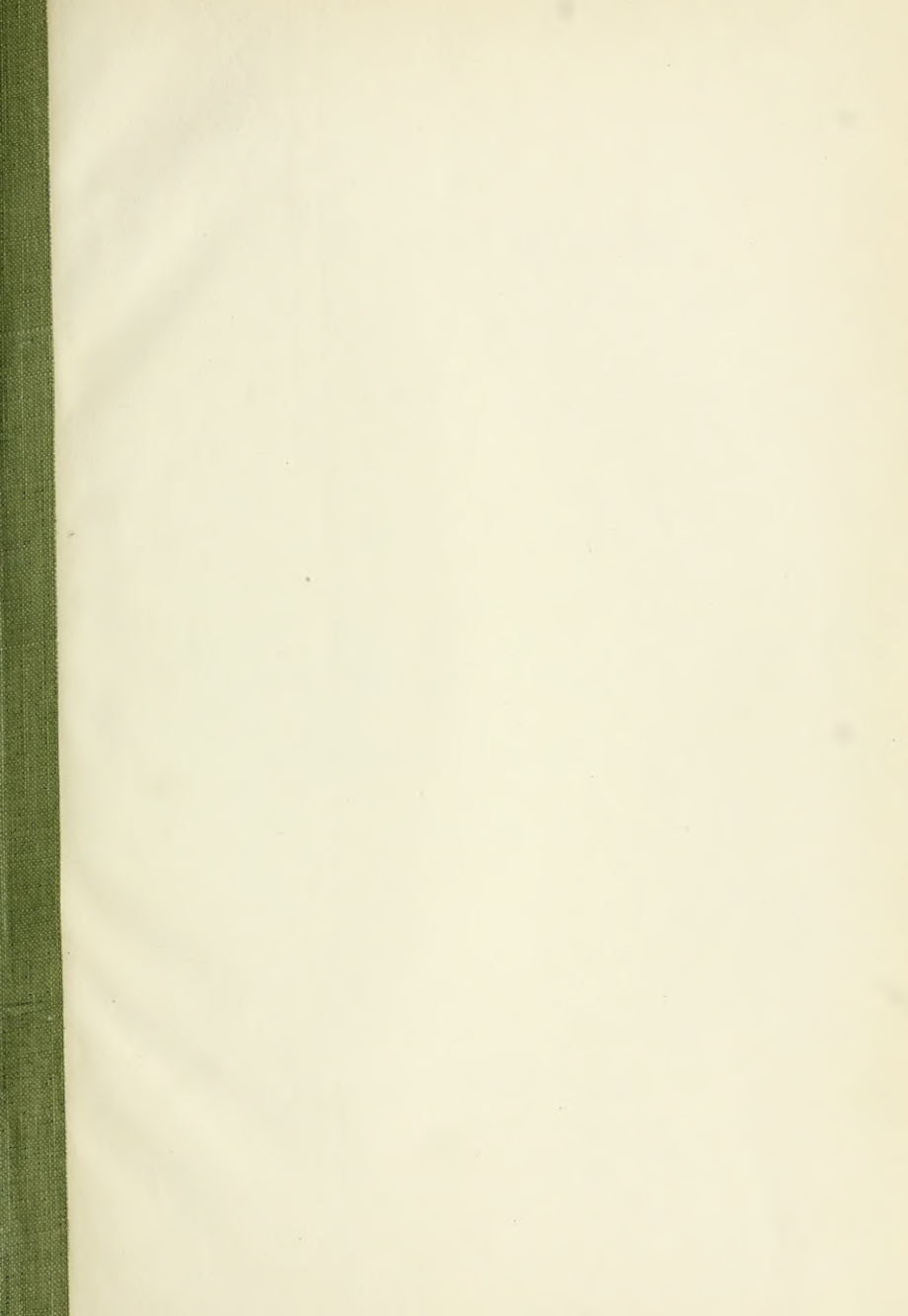
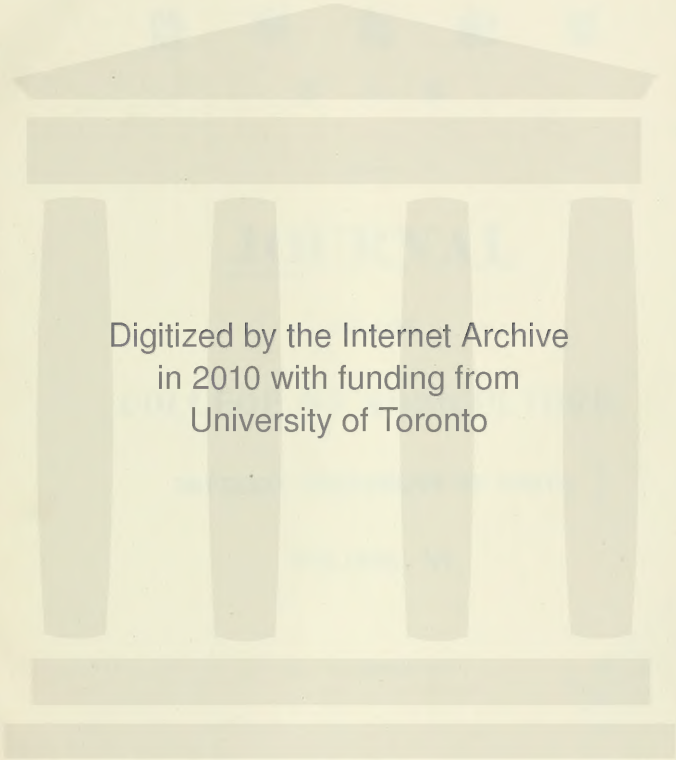


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Ueber das Maximalwachstum der japanischen Holzarten.

VON

Prof. Dr. **Seiroku Honda.**

Mit Tafel I—IV.

Als Beitrag zur Förderung des Forstwesens und der Naturdenkmalfrage unternahm ich es, schon seit etwa 20 Jahren, die existierenden, historisch bekannten Riesenbäume in Japan und den Kolonien zu beschreiben. Dank dem freundlichen Bemühen vieler Herren, die im ganzen Lande forstlich tätig sind, konnte ich im vorigen Jahre eine Menge Berichte darüber erhalten. Die in Frage kommenden Exemplare erreichten im ganzen die Zahl 5922. Von diesen wählte ich 1500 Exemplare aus und beschrieb sie in einem Büchlein „Dai Nippon Roju-Meibokushi“ (Forstlich und historisch bekannte Riesenbäume in Japan), welches kürzlich (im Dezember 1913) in Tokio veröffentlicht wurde. Die folgenden Zeilen sind dem Büchlein entnommen. Da aber diese Arbeit noch unvollkommen ist, hoffe ich sie später zu vervollständigen.

I. JAPANISCHE BÄUME NACH IHRER GRÖSSE :

(Japanischer Name)	(Lateinischer Name)
1. Kusunoki.....	<i>Cinnamomum Camphora</i> Nees et Eberm.
2. Sugi	<i>Cryptomeria japonica</i> Don.
3. Taiwansawara	<i>Chamaecyparis formosensis</i> Matsum.
4. Ichōnoki	<i>Ginkgo biloba</i> L.
5. Shii	<i>Pasania cuspidata</i> Oerst.
6. Matsu	<i>Pinus Thunbergii</i> Parl. und <i>P. densiflora</i> S. et Z.
7. Mukunoki	<i>Aphananthe aspera</i> Planch.
8. Katsura	<i>Cercidiphyllum japonicum</i> S. et Z.

9. Keyaki *Zelkova serrata* Mak.
10. Byakushin *Juniperus chinensis* L.
11. Linju *Sophora japonica* L.
12. Yamazakura *Prunus donarium* Sieb.
13. Tochinoki *Aesculus turbinata* Blume.
14. Sendan *Melia japonica* G. Don.
15. Inoki *Celtis sinensis* Pers.
16. Gazumaru *Ficus retusa* L. var. *nitida* Miq.
17. Shioji *Fraxinus Sieboldiana* Bl. var. *sambucina* Bl.
18. Tabunoki *Machilus Thunbergii* S. et Z.
19. Momotamana *Terminalia Catappa* L.
20. Konara *Quercus glandulifera* Bl.
21. Sawara *Chamaecyparis pisiferu* Endl.
22. Hinoki *Chamaecyparis obtusa* S. et Z.
23. Kaya *Torreya nucifera* S. et Z.
24. Yanagi *Salix* sp.
25. Kashiwa *Quercus dentata* Thunb.
26. Momii *Abies firma* S. et Z.
27. Maki *Podocarpus macrophylla* Don.
28. Kunugi *Quercus serrata* Thunb.
29. Kashi *Quercus* sp. (immergrün.)
30. Yachidamo *Fraxinus mandshurica* Rupr. var. *japonica* Max.
31. Karamatsu *Larix leptolepis* Gord.
32. Harunire *Ulmus campestris* Sm. var. *luevis* Planch.
33. Harigiri *Kalopanax ricinifolius* Miq.
34. Akushide *Carpinus laxiflora* Bl.
35. Aogiri *Ferniana plataniifolia* R. et Br.
36. Akagi *Bischofia javanica* Blume.
37. Muro *Juniperus rigida* S. et Z.
38. Kuri *Castanea sativa* Mill.
39. Kaele *Acer* sp.
40. Saikachi *Gleditschia horrida* Mak.
41. Mochinoki *Ilex Othera* Spreng.
42. Ihiba *Thajopsis dolabrata* S. et Z.
43. Horutonoki *Elacocarpus decipiens* Hemsl.
44. Koyanuki *Sciadopitys verticillata* S. et Z.
45. Todomatsu *Abies sachalinensis* Mast.
46. Nagi *Podocarpus Nageia* R. Br.
47. Kuwa *Morus* sp.
48. Kuroganemochi *Ilex rotunda* Thunb.
49. Mukuroji *Sapindus Mukurosi* Gaertn.

II. DIE GRÖSSTEN EXISTIERENDEN BÄUME IN JAPAN :

(Umfang an 1.5 m Höhe).

1. *Gamō-no Ōkusu*, der Riesen-Kampferbaum (*Cinnamomum Camphora*) von *Gamō*. Auf *Gamō*, *Aira-Gun*, *Kagoshima-Ken*. Umfang 22.4 m; Höhe 27 m; Alter 800 (Taf. I).

2. *Kamishiroi-no-Ōkusu*, der Riesen-Kampferbaum (*Cinnamomum Camphora*) von *Kamishiroi*. Auf *Kamishiroi*, *Chikujō-Gun*, *Fukuoka-Ken*. Umfang 21.8 m; Höhe 18 m; Alter 1800 (Taf. II).

3. *Takaoka-Shichihonsugi*, die siebengabelige Kryptomerie (*Cryptomeria japonica*) von *Takaoka*. Auf *Suhiro-Chō*, *Takaoka-Shi*, *Toyamä-Ken*. Umfang 20.0 m; Höhe über 36 m; Alter über 1000 (Taf. III).

4. *Arisan-no-Shinboku*, der heilige Baum von Arisan (*Chamaecyparis formosensis*). In *Arisan*, *Kagichō*, Formosa. Umfang 19.7 m; Höhe 40 m; Alter 2000 (Taf. IV).

Dazu kommen noch folgende kleinere Exemplare vor:—je 1 *Ginkgo biloba* und *Pasania cuspidata* mit 19.4 m Umfang; 1 *Cinnamomum Camphora* mit 19.1 m Umfang; je 1 *Cryptomeria japonica* und *Cinnamomum Camphora* mit 18.8 m Umfang; 2 *Cinnamomum Camphora* mit 18.2 m Umfang; je 4 *Cryptomeria japonica* und *Ginkgo biloba*, 2 Kiefern, je 1 *Aphananthe aspera*, *Cinnamomum Camphora*, *Cercidiphyllum japonicum*, *Zelkova serrata* und *Juniperus chinensis* mit 17.6 m—15.2 m Umfang.

III. DER STÄRKSTE EXISTIERENDE BAUM:

Gamō-no-Ōkusu mit 22.4 m Umfang (Taf. I).

IV. DER HÖCHSTE EXISTIERENDE BAUM:

Kryptomerie in Forstrevier Nagakizawa, Akita-Ken, von über 60 m Höhe.

V. DER ÄLTESTE EXISTIERENDE BAUM:

Arisan-no-Shinboku, 2000 Jahre alt. (Taf. IV).

VI. DIE RIESENBÄUME NACH IHRER ZAHL:

1. *Cryptomeria japonica*.
2. *Cinnamomum Camphora*.

3. Kiefer.
4. *Ginkgo biloba*.
5. *Zelkova serrata*.
6. *Prunus donarium*.
7. *Pasania cuspidata*.
8. *Quercus* sp. (immergrün).

VII. TABELLARISCHE ÜBERSICHT ÜBER DEN DURCHSCHNITTlichen MAXIMALWACHSTUM DER JAPANISCHEN HOLZARTEN. (Baumhöhe und Alter meist nach Schätzung.)

Nr.	Holzart	Zahl der Exemplare	Umfang an 1,3 m Höhe (m)	Höhe (m)	Alter
1	<i>Cinnamomum Camphora</i>	129	15-18 (selt. 22.1)	36 (selt. 45-56)	1000 (selt. 1800-2000)
2	<i>Cryptomeria japonica</i>	286	18 (selt. 20.0)	55 (selt. 61)	800-1000 (selt. 1000-2000)
3	<i>Chamaecyparis formosensis</i>	1	20	45	2000
4	<i>Ginkgo biloba</i>	96	15 (selt. über 18)	45 (selt. 62)	1000 (selt. 1500)
5	<i>Pasania cuspidata</i>	26	12 (selt. 19.4)	18 (selt. 27)	1000 (selt. 1500)
6	Kiefer	377	15 (selt. 16.4)	45	500 (selt. 1000-1500)
7	<i>Alnus aspera</i>	20	11-12 (selt. 15.8)	27 (selt. 45-56)	400-500 (selt. 800-1000)
8	<i>Cercidiphyllum japonicum</i>	16	9 (selt. 15.1)	27 (selt. 45)	500-600 (selt. 1000)
9	<i>Zelkova serrata</i>	119	15	27-36 (selt. 55)	1000
10	<i>Juniperus chinensis</i>	11	9-11 (selt. 15.1)	27	1000 (selt. 1600)
11	<i>Sophora japonica</i>	6	13	18 (selt. 45)	300-400
12	<i>Prunus donarium</i>	98	12	18	400-500 (selt. 1000-1800)
13	<i>Aesculus turbinata</i>	12	12	27-36 (selt. 45)	600-700 (selt. 1000)
14	<i>Melia japonica</i>	1	12	24	500
15	<i>Celtis sinensis</i>	16	8-9 (selt. 12.1)	18-27	400-500
16	<i>Ficus retusa</i> var. <i>nitida</i>	6	12	22	800
17	<i>Fraxinus Sieboldiana</i> var. <i>sambucina</i>	2	9	27	400-500
18	<i>Machilus Thunbergii</i>	7	9	18-27	500
19	<i>Terminalia Catappa</i>	1	11	11	400
20	<i>Quercus glauclifera</i>	2	9	18	700-800 (selt. 1000)
21	<i>Chamaecyparis pisifera</i>	4	9	55	500-600 (selt. 1000)

Nr.	Holzart	Zahl der Exemplare	Umfang an 1,5 m Höhe (m)	Höhe (m)	Alter
22	<i>Chamaecyparis obtusa</i>	12	9	55	1000
23	<i>Torreya nucifera</i>	15	9	36	1000
24	Weide	7	6 (selt. 9)	36	300-400
25	<i>Quercus dentata</i>	3	9	27	400-500
26	<i>Abies firma</i>	11	9	45	400-500 (selt. 1000)
27	<i>Podocarpus macrophylla</i>	5	6	27 (selt. 45)	500-600 (selt. 1000)
28	<i>Quercus serrata</i>	8	8	27 (selt. 36-55)	300-400 (selt. 900)
29	<i>Quercus</i> sp. (immergrün)	21	8	27 (selt. 36)	400-500
30	<i>Fraxinus mandshurica</i> var. <i>japonica</i>	15	8	27	300-400 (selt. 1000)
31	<i>Larix leptolepis</i>	1	8	36	400-500 (selt. 1200)
32	<i>Ulmus campestris</i> var. <i>laevis</i>	7	6	27 (selt. 36-45)	300
33	<i>Kalopanax ricinifolius</i>	11	7	18-27 (selt. 36)	300-400
34	<i>Carpinus laxiflora</i>	2	6	18	400-500 (selt. 1200)
35	<i>Firmiana platanifolia</i>	1	6	18	400
36	<i>Bischofia javanica</i>	1	6	18	250
37	<i>Juniperus rigida</i>	2	6	18	—
38	<i>Castanea sativa</i>	4	6	18-27	400-500
39	Ahorn	14	6	18-27	300-400 (selt. 1000)
40	<i>Gleditschia horrida</i>	4	6	18-27	300-400
41	<i>Ilex Othera</i>	5	6	18 (selt. 31)	400-500 (selt. 1000)
42	<i>Thujopsis dolabrata</i>	3	6	18	1000
43	<i>Elaeocarpus decipiens</i>	1	6	7	900
44	<i>Sciadopitys verticillata</i>	3	6	27 (selt. 51)	800 (selt. über 1000)
45	<i>Abies sachalinensis</i>	4	6	36	400
46	<i>Podocarpus Nagelii</i>	6	6	18	700-800
47	Maulbeere	7	5	18	300-400 (selt. 1000)
48	<i>Ilex rotunda</i>	2	5	9	300
49	<i>Sapindus Mukurosi</i>	1	5	18	300

ERKLÄRUNG DER TAFELN.

TAFEL I.

Gamō-no-Ōkusu, der Riesen-Kampferbaum (*Cinnamomum Camphora*) von Gamō. Umfang 22.4 m; Höhe 27 m; Alter 800.

TAFEL II.

Kamishiroi-no-Ōkusu, der Riesen-Kampferbaum (*Cinnamomum Camphora*) von Kamishiroi. Umfang 21.8 m; Höhe 18 m; Alter 1800.

TAFEL III.

Takaoka-Shichihonsugi, die siebengabelige Kryptomerie (*Cryptomeria japonica*) von Takaoka. Umfang 20.0 m; Höhe über 36 m; Alter über 1000.

TAFEL IV.

Arisan-no-Shin boku, der heilige Baum von Arisan (*Clamaecyparis formosensis*). Umfang 19.7 m; Höhe 40 m; Alter 2000.









Experimental Studies on the Embryonal Development in an Angiosperm.

By

Shunsuke Kusano.

With Plates V-IX and 28 Text-Figures.

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I. Introduction.

In reviewing a large amount of literature dealing with the embryonal development and the accompanying phenomena in Angiosperms, one would easily find that the cytological study and the experimental or field work are in most cases not well co-operated. For instance, a thorough recapitulation attempted by WINKLER ('08) on parthenogenesis and by TISCHLER ('12) on parthenocarp will amply show that many important data offered by experimental researches and field observations remain inexplicable for lack of cytological evidences. On the other hand, the cytology on the same subject concerns itself, in the vast majority of cases, with the cyto-morphological record, neglecting the experimental and physiological considerations. In going deeply into this matter no one would hesitate to place great importance upon

the combination of the two methods of study. In recent years the progress in our knowledge of plant breeding makes the need for researches in this way more urgent and pressing, but a deficiency of best-suited materials for the purpose appears to have greatly hindered our approach to the desired end.

At the close of my study (KUSANO, '11) on the mycorrhizal problem of *Gastrodia elata*, an ecologically interesting orchid, my attention has been drawn to the cytology of its generative sphere. The present study was taken up originally with a view to learn the normal course of the embryonal development, but during the progress of the work it fortunately came into my mind that the material suggested the possibility for experimental-cytological researches. Experiments prosecuted for a certain purpose, however, presented new problems one after the other, for the solution of which the scope of the experiments was necessarily extended so widely that the work done over a period of five years was yet far from completion. The subjects of special interest, with which I was so far concerned, were related to the occasional omission of the chromosome reduction and to parthenogenesis, parthenocarpy, and polyembryony. These problems are all very interesting and important, but are so complicated as to need further experimental studies, to bring forth results of greater weight than those arrived at at present. With the results so far accumulated, however, I will now endeavour to give a general view. In recording them in the present article I will not dismiss from consideration those points which are not yet settled satisfactorily, but which excite greater interest for further investigations. It is proposed first to give an account of the normal course of the embryonal development and then to consider the accompanying phenomena on the basis of experiments.

No record has hitherto been made on the cytology of the reproductive phase of *Gastrodia*, but on other orchids there are numerous investigations which have more or less bearing upon the present subject of study. They will be mentioned in the course of this paper.

II. Material and Methods.

As I have already stated elsewhere ('11), the flowering individual of *Gastrodia* is represented by a single hibernated tuber. Without any nutri-

tive communication with the surrounding medium, it develops towards the end of May a long inflorescence axis. Photosynthesis is quite lacking, and the flowers and fruits are produced exclusively by virtue of a limited amount of nutritive materials stored up in the tuber. On this account, the nutrition of the potted tuber can be maintained quite normally, when the latter is kept sufficiently moist.

In furnishing the material for study the flowering potted tubers were brought into a laboratory room, while the inflorescence axes were yet very short. The growth of the axes and the development of the flowers went on vigorously, and a few flowers opened every day in succession in each inflorescence. The normal course of development was studied on flowers pollinated on the day of bloom. For comparison, the development of flowers subjected to various kinds of treatment was followed out. After becoming acquainted with the finer details of the cytological features by means of the microtome, the fresh ovules in toto offered the cytological sequence during the embryo-sac development with comparative easiness and exactness, on account of the simple structure of their sporophytic portion. Especially, the presence of only a single cell-layer over the upper half of the embryo-sac, up to the time of fertilization, enabled me to take through it a clear view on the finer structure of the living sac. This advantage facilitated in great measure the observation of the successive stages in the gametophytic development on sufficient materials and accelerated the progress of the work. Regarding the important cytological features, however, I consulted microtome sections.

To the best advantage for the experiment the flower showed a strong resistance towards various kinds of treatment. When the upper portion of the ovary was cut away and the cut surface was sealed with paraffin, the remaining portion could continue the further development undisturbed. In this way I could study, when necessary, several succeeding stages of the ovular development in one and the same ovary. An abscised inflorescence axis or a piece of it, when the cut end was placed in water, kept the flower quite fresh and healthy, and allowed it to develop the mature fruit. Even abscised flowers, if kept from drying, could remain alive until maturation of the seed.

An unusually rapid embryonal development needed a hasty microscopical examination of the ovule in following out its successive stages, and it

greatly hindered us in making an extensive comparative study on a large number of flowers which were subjected at the same time to different conditions. The present work was, therefore, extended necessarily over several years. In this way I could fortunately repeat the experiments and observations to obtain confirmatory results on certain important points.

The study was chiefly made on *Gastrodia elata*, but a few tubers of a form, *viridis* Mak., also furnished material. This form is distinguished from the type by a blue greenish colour, smaller tuber, shorter and slender inflorescence axis, and smaller flower and fruit. However, as regards the embryonal development no distinction could be found between the two forms.

It seemed desirable to give in the present article the account of the reduction division in the microspore formation as well as in the megaspore. The material for the former has not, however, given a complete series of stages and a fuller account will be reserved for a future communication.

The material for studying finer nuclear features was treated by following the usual method: FLEMMING's mixture and acetic sublimate as fixing fluids, and FLEMMING's and HEIDENHAIN's methods for staining. In studying the general structure of the embryo-sac acetic alcohol was often used for the sake of convenience.

III. Normal Course of Development.

The account of the normal course of the embryonal development has a two-fold importance. First, it is to complete the life-history of *Gastrodia* in conjunction with a former paper (Kusano, '11), in which the vegetative sphere has been dealt with, and thus to substantiate our knowledge of the Orchidaceae regarding the relation between the generative and vegetative spheres. Secondly, it may offer a basis for the discussion of the mutual relation among different phases in the embryogeny, which shall be attempted on the experimental researches. In connection with the latter object the development of the fruit-wall and the seed-coat shall be treated in detail.

1. DEVELOPMENT OF THE EMBRYO-SAC.

While the ovule is yet rudimental, representing itself as a short cylindri-

cal process on the surface of the placenta, and consisting of a single axial row of cells surrounded by an epidermal cell-layer, the terminal cell of the row is found differentiated into an archesporium cell, being easily distinguished by its larger nucleus and more densely staining cytoplasm than in other cells. As in other orchids already investigated, the archesporium cell becomes directly the embryo-sac mother-cell, as may be seen at once from its nucleus reaching the synapsis stage.

Synapsis is attained in the usual manner. At the resting stage the chromatin and linin substances are distributed in the nuclear cavity without giving a definite structure. Entering the presynapsis stage, an aggregation of chromatin and linin takes place, giving rise to a coarse reticulum (Fig. 1). As indicating the approach of synapsis, the nuclear reticulum contracts away from the nuclear membrane and the meshes of the network become denser in the central portion (Fig. 2). During this process it appears to accompany the withdrawal of the nuclear substances along the thread into the central portion. When the contraction proceeds further, the chromatic bodies and linin substance are almost all drawn together in a close mass round or generally near the nucleolus. The mass gives first the appearance of an entangled thread structure, beset with a few linin strands extending to the nuclear membrane (Fig. 3). These strands disappear afterwards almost entirely, and at the same time the mass assumes a compact, nearly homogeneous consistence, being represented as the synaptic knot (Fig. 4). The knot often shows such a compactness and structure as to give the appearance of a large nucleolus, being, however, distinguished from the true nucleolus by a strong staining capacity.

Following this midsynaptic period an entangled thread system begins to emerge from the knot (Fig. 5). The gradual loosening of the threads gives rise to the spireme stage (Fig. 6). The loosening proceeds so far as to make a continuous homogeneous thread distribute itself in the whole nuclear cavity, chiefly running along the inner side of the nuclear membrane (Fig. 7).

The construction of chromosomes from the spireme thread involves numerous phases, whose serialiations and interpretations are so difficult that several controversial views yet prevail. In *Gastrodia*, I believe to have obtained serial stages so complete as to understand the main feature of the

chromosome formation correctly. However, to eliminate misunderstanding of events, the account will be reserved until supported by the facts furnished by an exact count of the number of chromosomes in the subsequent nuclear generations up to the stage of the embryo-sac formation and in somatic cells. In this place I shall pass to the general feature of divisions implied in the formation of the embryo-sac from the archesporium.

The first division. Previous to the spindle formation, the nucleus of the archesporium lying first in the centre of the cell cavity is displaced a little towards the micropylar end (Fig. 24), so that the upper pole of the longitudinal spindle often comes close to the cell-wall, while the lower pole approaches the centre of the cavity, to be surrounded by a dense mass of cytoplasm (Figs. 40, 42, 48). The departure of the daughter chromosomes from the equatorial plate for the pole is usually simultaneous (Figs. 44-48), but often an irregular distribution of the chromosomes on the spindle is observed at this stage. At the anaphase the displacement of the spindle becomes so apparent, that the chromosomes arrived at the upper pole often dash against the cell-wall, while those at the other pole are found occupying nearly the central position of the cell cavity (Figs. 51, 52). So long as the chromosomes are represented distinctly at the late anaphase, there can be found no difference in the phase of both poles. Entering the telophase stage, the process of reconstruction of both daughter nuclei proceeds somewhat differently; while the anastomosing and dissolving chromosome mass begins to enlarge in the lower pole, it remains smaller and compact in the upper (Figs. 51-53); sometimes, while the nuclear membrane appears on the lower, the upper mass remains naked. A rapid increase of the lower nucleus in size makes the different phases in both nuclei more prominent (Figs. 52, 53). Sooner or later the upper nucleus is compressed against the cell-wall, while the lower one grows to the full size (Fig. 54).

The spindle fibres fade away centripetally from both poles, and at the time the lower nucleus is nearly completed the residual fibres are yet recognized between both nuclei. Throughout their median portion a cell-plate is laid down, and its peripheral extension divides the cytoplasm into a lower larger and an upper smaller half. During the fading of the spindle fibres we recognize a decrease of distance between the daughter nuclei, which is

enhanced by a rapid enlargement of the lower nucleus, especially in its longitudinal direction. The upper cell receives an exceedingly small amount of cytoplasm (Figs. 52-54), and is at first platy, becoming soon after crescent from compression by the enlarging sister-cell. The crescent upper cell undergoes sooner or later disintegration; the nucleus almost without any growth disorganizes and assumes a coarse granular structure with an indistinct outline (Fig. 56), and finally the whole protoplast is converted into a hyaline mass, heavily stainable with safranin and haematoxylin (Figs. 57, 58). In the mean time the nucleus of the lower cell attains to maximal size. At first it assumes an oval form with longer axis longitudinal to the ovule (Fig. 54); later it becomes spherical and occupies just the same position as its mother nucleus.

Frequently I could observe a split of the daughter chromosome at the anaphase stage (Fig. 49). However, in the majority of cases such a doubling of chromosomes could not be ascertained. Also the late telophase frequently gives a clear interkinesis, whereas the chromosome takes on an x-shape (Fig. 52).

The second division. In the lower daughter nucleus it is not easy to point out the real resting stage. The interkinesis is followed by the alveolation and dissolution of chromosomes (Fig. 53). When the reticulum appears, a certain number of small globules of a nucleolar nature come in view (Fig. 54). They are mostly similar-sized, though a few are far smaller. From the number, ranging approximately from 16 to 20 or thereabout, which corresponds nearly to the number of chromosomes, they may appear as if to represent the chromosomes in dispersal of their substance. The staining character, however, differs from the chromatic bodies. At about a similar stage I could find the nuclei containing one or two large typical nucleoli. Whether both represent quite the same stage, or if not, which of both stages precedes the other, is not clear.

Following the reticulum stage, I could not find a continuous spireme thread. On the other hand, nuclei containing a nucleolus and chromosome segments in the peripheral arrangement came frequently under my observation (Fig. 55). A somewhat irregular form of the segments, joined to each other with delicate linin thread, suggests their origin as not by segmentation

of a single spireme thread, but as condensation of the chromatic material from the reticulum. From a few countings the number of the segments appears to correspond approximately to that of the diploid chromosomes or their split number. With the disappearance of the nucleolus and the nuclear membrane the segments become more definitely shaped and distinct. In the figure, so far studied, the somatic number of chromosomes are counted* (Figs. 56, 57). The nucleus becomes elongated a little in longitudinal direction, with coarse fibres running freely in the same direction. The chromosomes retreat in the centre of the cavity in a more condensed form and as curved rods (Fig. 58).

After the formation of the spindle the general mitotic features are quite similar to those of its mother nucleus: the spindle lies nearer to the micropylar end, the cytoplasm assembles more densely around the lower pole, etc. Also, as in the mother nucleus, we get in the late anaphase similar groups of daughter chromosomes at both poles (Fig. 63); and entering the telophase the upper group remains almost without increasing in size, while the lower one shows conspicuous enlargement with the precipitation of the membrane round it (Fig. 69). By the second division of the archesporium we get daughter-cells quite similar to those resulting from the first division, the lower larger cell with a rapidly enlarging nucleus and the upper smaller cell. The latter comes soon to obliteration and forms the second, hyaline crescent cup (Fig. 70).

The two divisions of the archesporium represent the tetrad divisions, whereby we get here two ephemeral and one functional cell. The latter is the megaspore, or the embryo-sac-cell, which gives rise to an embryo-sac by further divisions (Fig. 70).

A noteworthy fact during the tetrad divisions is, that the upper daughter-cell on each division scarcely receives any amount of cytoplasm, giving the indication of saving the cytoplasm in the archesporium for the functioning megaspore. This conduct expresses clearly the significance of the division as a reduction of the nuclear contents, in full accordance with the formation of polar bodies during the maturation of egg-cells in animals.

Embryo-sac. The megaspore nucleus, passing through the resting stage (Fig. 70), goes on to division. Condensation of the chromatic bodies and linin threads results in the formation of the spireme segments. Perhaps no

* The chromosome number will be given later on.

single spiroeme thread has been formed previously. They are irregular in outline and connected by fine threads to each other. They represent the chromosomes. The broader bands, which occur frequently, may show an early stage of the chromosome segments. After the chromosomes become more compact (Fig. 71), they range themselves irregularly, with the disappearance of the nuclear membrane and nucleolus, on the multipolar spindle fibres (Fig. 72). Becoming bipolar, the spindle lies centrally and longitudinally, accompanying at both poles the same quantity of cytoplasm (Fig. 73). Thus differing from the previous two divisions, the megaspore is divided into two equal halves (Figs. 74, 75). The daughter nuclei lie in a symmetrical position in the cell cavity (Fig. 76). At the time of reconstruction of the daughter nuclei the spindle fibres are found persistent between both nuclei (Fig. 77). Owing to the enlargement of the cell the cytoplasm becomes more vacuolate on the lateral side of the persisting fibres. Even after the disappearance of the fibres there can be seen a string or strings (Fig. 78) of cytoplasm as a median connecting strand between the plasmic masses assembled round the upper and lower nuclei.

In this division no cell-plate is formed on the fibres, so that the daughter nuclei lie freely in a common cell cavity and enter into the resting condition with a prominent nucleolus and a usual reticulum structure.

Owing to the further enlargement of the cell cavity the enlarging vacuoles come ultimately to fuse together.

According to the arrangement of cytoplasm and vacuoles at the binucleate stage of the sac, the features of the further development present certain variations. As showing the typical course of development, two daughter nuclei, lying at the opposite end of the cell and being surrounded by an equal quantity of cytoplasm, grow up equal in size (Fig. 78), and at the time each nucleus undergoes division, the upper and lower halves of the cytoplasm are yet connected by the median plasmic string. Under this condition both spindles, presenting nearly the same stage and acquiring nearly the same size, lie at right angle to each other, the upper being transverse to the long axis of the sac, while the lower being longitudinal. Such an orientation of two spindles is warranted when they happen to lie in a common mass of cytoplasm (Figs. 80, 84), which appears to occur when the cytoplasm is richer in amount.

More frequently, a large vacuole occupies the central position of the cell cavity, and the protoplast is divided into the upper and lower halves, being only connected by a thin plasmic membrane along the cell-wall. In this case the direction of the lower spindle is mechanically hindered to orientate itself longitudinally, that is, at right angle to the upper one (Figs. 79, 81, 82, 83, 87).

With the succeeding two divisions of the megaspore nucleus we get four nuclei. No further division takes place and thus the embryo-sac is organized with four nuclei.

In forming a complete sac one of the sister nuclei at the charazal end—the upper one when the direction of division is longitudinal—traverses towards the micropylar end and comes to be embedded, together with the two nuclei at the micropylar end, in a common mass of cytoplasm (Figs. 93–95). Later, the limiting plasmic membrane is precipitated between each two nuclei (Fig. 95), often preceded by the formation of the fibres (Figs. 93, 94). The original upper two nuclei are organized into the synergids, and the one coming from the charazal end forms the egg-cell. The remaining charazal nucleus lies freely in the sac, being surrounded by a small quantity of cytoplasm left behind during the formation of the egg-apparatus, and functions as the pole nucleus of the sac. Afterwards it is often found attached to the egg-apparatus (Figs. 97, 98).

When a large central vacuole is present in the sac, the charazal nucleus forming the egg traverses upwards peripherally along the plasmic membrane surrounding the vacuole (Figs. 94, 95).

At the binucleate stage of the sac I often observed the unequal gathering of cytoplasm at both ends of the sac, the latter being separated by a large central vacuole, with always a lesser amount in the lower (Figs. 82, 83). Sometimes the unequal distribution proceeds so far as to leave the lower nucleus in a thin plasmic layer, or, in extreme cases, almost nakedly along the cell-wall (Figs. 86–89). Under this condition the growth of the lower nucleus is much disturbed, and its division gives rise to a smaller spindle (Figs. 87, 88), placed necessarily in transverse direction, that is, in a plane parallel to that of the upper spindle. The resulting daughter nuclei are consequently much smaller (Fig. 85). In this case the egg-forming nucleus is smaller than the synergid ones. However, in organizing into an egg-cell

it may become equal to, or exceed, the synergid nuclei in size. When an extremely small amount of cytoplasm is left on the charazal end, the nuclear division takes place with difficulty, and thus the complete organization of the embryo-sac would be scarcely possible. Two sister nuclei are then exceedingly small and perhaps unable to separate from each other. It is almost customary that they lie in close contact (Fig. 91). Careful examination shows that they may in some cases fuse together (Figs. 90, 91). As showing the fusion we obtain dumb-bell-shaped, rarely spindle-shaped nuclei, provided with two distinct nucleoli. I could not find a two-nucleolate nucleus which assumed a spherical form as showing a further stage of fusion, though it might possibly occur. The serial stages I reproduced (Fig. 91) for fusion may also point out the reversal process, that is, amitotic division. However, as spindle figures are found commonly at the corresponding place of the sac, the occurrence of amitosis appears rather improbable.

As an extreme case we find an abnormal formation of the lower spindle from the naked charazal nucleus. At the anaphase the daughter chromosomes give an impression as if about to dissolve into a more or less homogeneous mass (Fig. 88). While it is on the way to the pole, the spindle fibres become faint, and two homogeneous masses are left behind (Figs. 89, 92). The formation of the functional daughter nuclei is in this case unsuccessful.

All these abnormal nuclear features appear to be connected with a small amount of the surrounding cytoplasm, in consequence of, I believe, an unfavourable nutritive condition. The fusion of the daughter nuclei will be ascribed to their hungry condition, as can be seen in the instances given by NEMEC ('10), BONNET ('12) and others (TISCHLER, '12, p. 20).

I had no occasion to make an extended observation and study how an incomplete development of the charazal nucleus is induced. So far as observed, it appears a fact that, when the amount of cytoplasm is relatively smaller at the binucleate stage, the cell cavity becomes occupied by a correspondingly larger central vacuole, whereas the cytoplasm is extended into a thin plasmic membrane around it, while its main portion is massed at the upper pole. Such an arrangement of cytoplasm can be seen in flowers developing under ordinary conditions, yet it occurs more frequently when the flower is subjected to unfavourable nutritive conditions.

2. FERTILIZATION.

The pollen-tube, after generally more or less torsion, finds its way through a single layer of the nucellular cells into the embryo-sac, and its tip advances a short distance between the cells of the egg-apparatus. The foremost nucleus in the tube is slightly larger than those immediately following it; it represents the tube nucleus. In a microtome section I could find that the wall of the tube gave an appearance at its apex as if gelatinized for discharging the tube contents (Fig. 98). As to whether the tube discharges into one of the synergids or outside it, I could not determine exactly. But as showing the discharge, there appears an hyaline mass corresponding in size to the synergid. In fresh material it looks like a gelatinized substance and in fixed material it is characterized by taking on safranin or haematoxylin heavily. The consistence is not homogeneous, and we can point out usually one or two denser spherical portions (Figs. 99-101). This mass may often show a connection with the disintegrated tube-wall. From the size the mass may be taken as having originated from the contents of the tube and synergid, as usually assumed.

The fusing stage of the sexual nuclei was observed in many living ovules, but the successive processes of fusion could not be followed out in one and the same ovule. The male nuclei, which have been oblong in the tube, take an oval or spherical form during the movement towards their mate nuclei. The male nucleus entering the egg-cell can be distinguished from the female by its smaller size (Figs. 99, 100). The other male nucleus migrates downwards and approaches the pole nucleus with which it is to fuse (Fig. 101).

There occurs a triple fusion in the pole nucleus, a male nucleus, and a synergid nucleus. The latter two nuclei do not differ either in size or in form, so that which of them fuses earlier with the pole nucleus is scarcely possible to determine. The occurrence of the triple fusion is better shown by the fact that three nuclei come closely together (Fig. 101), and only a single nucleus is found at that place at later stages. It may be remarked in this connection that a marked deformation of the pole nucleus or the presence of more than one nucleolus in it cannot be taken as an indication of its fusion

with both a male and synergid nuclei, or with one of them. In my opinion this character of the pole nucleus points to its high activity concerning the nutritive function, which can be seen even previous to the entry of the pollen-tube (Fig. 95) or to fertilization (Fig. 98).

A quite similar fusion has already been reported by PACE ('07) in *Cypripedium*, which is characterized by having the 4-nucleate embryo-sac. In the similarly reduced sac in some Onagraceae, however, GEERTS ('09) and MODILIEWSKI ('09) did not ascertain the fusing action of the synergid nucleus. The triple fusion occurring in the 4-nucleate embryo-sac of orchids may show the same significance as in the usual 8-nucleate embryo-sac. But the morphological dignity of the fusing nuclei is not the same in both; in the latter sac one is the male, the other two are the upper and lower pole nuclei, while in the former sac one of the pole nuclei is replaced by a synergid nucleus. It may also be noted that, so far as observed in *Gastrodia*, the fusing affinity is not exhibited between the synergid and pole nuclei, in case the sac must remain long unpollinated or unfertilized, while in the 8-nucleate embryo-sac the upper and lower pole nuclei may fuse generally previous to fertilization. For securing fusion the synergid nucleus must be liberated from its cell. Perhaps this conduct of the synergid would be induced in connection with the discharging action of the pollen-tube.

Generally the triple fusion takes place later than the fusion between the sexual nuclei. It is often the case that three nuclei remain closely together but unfused in the sac, in which the elements of the egg and male nuclei have already mingled (Figs. 101, 102). As showing a great delay of the triple fusion we find the pole nucleus in contact with a smaller nucleus (male or synergid nucleus), when the fertilized egg has undergone the first division (Fig. 105). Such a case seems to show the fact that the double fertilization does not take place, as ascertained in some orchids by NAWASCHIN ('00) and STRASBURGER ('00). But a careful examination of the embryo-sacs, in which the embryo has grown to a few-celled stage disproves it, since a single nucleus is invariably present as a fusion product of the three nuclei. This was ascertained not only in microtome sections but most distinctly in the living material *in toto*, in which the clearness of view eliminated all chances of error of observation.

3. THE EMBRYO AND THE ENDOSPERM NUCLEUS.

Soon after fertilization the egg-cell elongates into a club-shaped body with the protoplast accumulating on the apical portion. A transverse division takes place and results in the formation of the apical compact and basal larger cells (Fig. 105). The successive divisions in the same direction produce a longitudinal row of cells representing a proembryo. Its apical cell then divides longitudinally, and from then on the division taking place in both directions increases the number of cells till an oval embryo results.

Without differentiation of the body organ the embryo can attain maturity as an oval, multicellular mass as in other orchids. The mature embryo appears in the fresh state pale yellow, packed with granular reserve materials, of which starch is an essential component. Before maturation of the embryo the suspensor shrinks and is found attached to the pointed end of the embryo as a brownish remnant. A hyaline mass originated from the pollentube and synergid persists until the embryo has nearly completed its development.

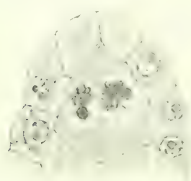
The mitotic division in the embryo proceeds similarly as in other somatic cells. In the prophase thick coiled spiremes appear and they give rise to the chromosome segments, 16 in number (Figs. 103, 104). The chromosome assumes the form of a longer or shorter rod.

Throughout the development of the embryo the endosperm nucleus remains undivided and in the resting condition, being surrounded by a small quantity of the radiating cytoplasm. Unless compressed between the growing embryo and the wall of the sac, as is often the case, it shows, unlike the pole nucleus, no deformation, and assumes a spherical form. It persists as such till the embryo attains the maximal size.

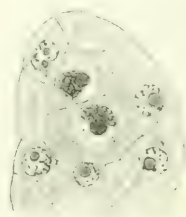
In a similarly derived endosperm nucleus of *Cypripedium* PACE ('07) mentioned several dividing stages and traced up its division into four nuclei. She showed also an embryo-sac in which the embryo, much advanced in development, accompanied three disintegrated nuclei. According to her, they are not the endosperm nuclei, but those for triple fusion, to which, however, they do not succeed. In the 4-nucleate embryo-sac of the Onagraceae the endosperm nucleus, which is the product of fusion of a pole and male nuclei,

divides to form an ephemeral endosperm. With *Gastrodia*, devoid of endosperm, occurrence or non-occurrence of the triple fusion seems to be indifferent for the nutrition of the embryo, but, unlike *Cypripedium*, it is almost universal that the fusion takes place to produce an undivided endosperm nucleus.

The pole and endosperm nucleus can be distinguished by the difference in their activity. The pole nucleus, as soon as the embryo-sac is completely organized, is thrown into a marked deformation, increases its chromatic contents, and contains 2-3 nucleoli, as already noticed. We assign to these characters a similar activity as exhibited by the antipodal cells in some of the usually organized embryo-sacs, which have been assumed as participating



Text-fig. 1. An ovule containing two archesporium cells side by side. $\times 900$.



Text-fig. 2. An ovule containing two archesporium cells one above the other. $\times 900$.

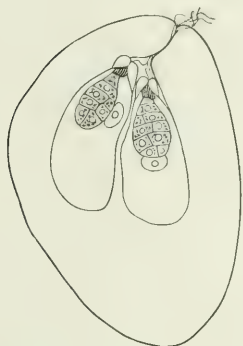
in the nutritive function. On the other hand, the endosperm nucleus is always spherical and in resting condition.

4. ABNORMAL FORMATION OF THE EMBRYO-SAC.

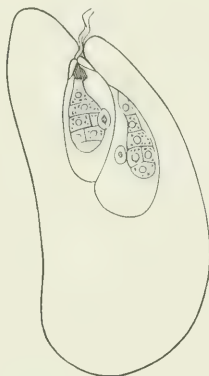
Some ovules contain two archesporium cells, lying sometimes side by side (Text-fig. 1) and sometimes one above the other (Text-fig. 2.) The development of their nuclei is precisely at the same stage. That both cells represent the archesporia is evidenced by the nuclei assuming synapsis.

The occurrence of two sporogenous cells in one ovule was observed by PACE ('09) in *Calopogon*. In *Gastrodia* it is not so common as reported by her, but I was able to find a few of such ovules on each slide prepared from some ovaries. At the synapsis stage or thereabout two cells are limited by a single membrane between, but not by the mucellular tissue as found by PACE, in some cases, in *Calopogon*.

The sequence of the archesporium cells during the formation of the embryo-sac could not be followed out further than the first nuclear division. Similarly, PACE did not mention the stages further than the megaspore formation. However, while examining the ovules *in toto* advanced for the seed formation, I often found the ovule with two embryo-sacs, each containing a young embryo. It is evident that two archesporium cells have undergone



Text-fig. 3. A young seed with two embryos in sacs lying side by side (9 days after pollination). $\times 150$.



Text-fig. 4. A similar seed with two sacs lying one above the other (10 days after pollination). $\times 150$.

normal development. In one case the sacs arrange themselves side by side (Text-fig. 3). Each micropylar end is surrounded by intact nucellar cells in the usual fashion. Two sacs receive independently pollen-tubes, and as an indication of fertilization a hyaline mass is found in each sac, attached to the suspensor. Being provided with an endosperm nucleus, each sac exhibits a quite normal inner structure. The compressed nucellar tissue is interposed between both sacs. Certainly, the two sacs have genetic connection with two archesporium cells lying side by side.

In the other case two sacs are arranged one above the other (Text-fig. 4) being limited by a membrane between. While in the upper sac the upper end occupies the position just below the micropyle, being surrounded by the typical nucellar cells in the usual way, it lies in the lower sac far

below on the lateral side of the upper sac, and is in direct contact with the compressed mucellular cells. Each sac is provided with a few-celled embryo and an endosperm nucleus. As a remarkable fact, only a single pollen-tube is found to pass through the micropyle. Fertilization of the upper egg is clearly demonstrated by the entry of the tube into the sac and by the presence of a hyaline mass in connection with the tube. How the lower egg derives the embryo remains in question. For in two or three ovules with the structure under consideration, which I was able to observe, the entry of the pollen-tube into the lower sac is by no means ascertained. Moreover, the position of the sac and the compactness of the ovular tissue overlying it lead us to the view that the sac does not receive a special pollen-tube. Hence it may be provisionally assumed that the egg undergoes parthenogenetic development or it is fertilized, though less probably, by one of the male nuclei set free in the upper sac and entering the lower one through the limiting membrane between.

5. CHROMOSOME NUMBER AND REDUCTION DIVISION.

After studying karyokineses throughout all stages in the embryonal development and noticing the number of chromosomes in the gametophytic and sporophytic tissues of the ovule, I am now in a position to consider in detail the manner of reduction division. During nuclear divisions, through which the embryo-sac is formed from the archesporium, I found in a large number of ovules that the number of chromosomes was double that of others, pointing to the omission of the chromosome reduction. As this is a most important matter closely bearing upon the ovular development, I have paid special attention to determine the exact number of chromosomes. Careful countings have been made on the spindle figures or equatorial plates in side, oblique, or polar view, carefully noting the occurrence of a longitudinal split of the chromosome.

Consulting the archesporium, its daughter-cells, the megaspore, the embryo-sac at the two- to four-nucleate stages, the embryo at the two- to many-celled stages, and also epidermal as well as funicular cells of the ovule, the diploid number of chromosomes was determined to be 16 or rarely 18, and consequently the haploid number to be 8 or 9. The subject of considera-

tion in this place is that the dividing figures of the gametophyte show both the haploid and diploid number of chromosomes.

Beginning with the archesporium the diploid number was ascertained to occur throughout almost all successive nuclear generations in the gametophyte. The relative number of ovules whose gametophyte contains the diploid chromosomes could scarcely be estimated with precision, since the required nuclear features for counting chromosomes were not presented at once in all the ovules. For the same reason it was difficult to confirm whether individual variations occurred among ovaries regarding the proportion of the number of the haploid and diploid ovules or not. But it will be seen from the following table that a large number of ovules develop the diploid egg (see Table I).

Table I.

Showing the number of ovules in which the number of chromosomes was estimated during the development of the gametophyte¹.

Nuclear generation.	Karyokinetic stage.	Number of ovules	
		Haploid	Diploid
1st generation (archesporium nucleus)	Chromosome formation	0	∞^2
" " " "	Equatorial plate	38	21
2nd " (archesporium to its daughter cell)	Anaphase to metaphase of the next division	16	15
3rd " (daughter cell to megaspore) ..	"	3	8
4th " (megaspore to 2-nucleate sac) ..	"	8	3 ³
5th " (2- to 4-nucleate sac)	Anaphase	4	1
Embryo		0	6 ⁴

At later nuclear generations I encountered difficulties in finding sufficiently clear figures which presented each chromosome distinctly. Nevertheless, it is

1. In the table are given only those ovules which presented such clear nuclear figures that they enabled me to determine the exact number of chromosomes.

2. More than a hundred.

3. In two other figures showing the equatorial plate approximately 16 chromosomes were found; they appeared to represent the daughter halves of the haploid chromosomes.

4. Further, I found 32 chromosomes in two other figures showing the equatorial plate in polar view, appearing to represent the daughter halves of the diploid (16) chromosomes.

evident, as the table shows, that the megaspore nucleus may contain the diploid chromosomes, and it distributes the same number of chromosomes into its daughter nuclei. It can be imagined that the diploid megaspore is unable to develop a complete embryo-sac, obliterating itself at a certain subsequent nuclear generation. In fact I could often observe the disorganization of the embryo-sac at the four nucleate stage, but it scarcely occurred earlier than this stage. The disorganization appeared to present individual variations according to different ovaries, so that in some it occurred more frequently, while in others nearly all the ovules developed quite healthy. At present I have no evidence to prove an incapability of the diploid megaspore for completing the embryo-sac.

A certain individual difference of plants in external appearance¹ attracted our attention as to whether there was an individual variation in the chromosome number, the diploid number being 32 in some and 16 in other stocks. However, it was disproved by the fact that somatic cells contained invariably 16 or 18 chromosomes showing the diploid number, and in one and the same ovary both 16 and 8 chromosomal nuclei were found in the gametophytes.

These facts would sufficiently justify the conclusion that certain ovules derive the embryo-sac at the omission of reduction division.

We now come to inquire whether the plan of the first mitosis in the embryo-sac mother-cell is fundamentally different, according to whether the chromosome reduction takes place or not. On examination of innumerable ovules it appears that all mother-cell nuclei pass through synapsis. As shown in the adjoining table (Table II), two days before bloom, more than one half of the nuclei in an ovary attain to the midsynapsis stage (knot), while the rest are yet at the presynapsis stage (reticulum stage). On the next day, that is, a day before bloom, postsynapsis predominates midsynapsis. It is almost certain that midsynapsis was attained at this period by those nuclei which had been at the presynapsis stage on the preceding day. Again, on the day of bloom, a certain number of nuclei is still found to be at the midsynapsis stage; they might have been at the presynapsis stage on the

1. Some tubers develop somewhat pale-coloured, smaller flowers upon slender inflorescence axes.

preceding day or days. Such a numerical relation of several nuclear stages on successive days may also be presented, if the presynaptic reticulum is converted directly into the spireme without passing through synapsis; but as no stage was ever found, which might show the spireme thread to have been derived following the usual process of the somatic mitosis, it may rather favour the view that the division in all mother-cell nuclei begins with such a plan as characterizes the heterotype mitosis.

Table II.

Showing in each ovary the relative number of different mitotic phases of the archesporium nuclei; the ovules were taken from a few sections of each ovary.

Nuclear stage	Period.								
	Two days before bloom			One day before bloom			The day of bloom		
	Ovary I	Ovary II	Ovary III	Ovary I	Ovary II	Ovary III	Ovary I	Ovary II	Ovary III
Presynapsis (reticulum) ..	223 (44.8%)	75 (15.0%)	116 (23.9%)	0	0	2	0	0	0
Midsynapsis (knot)	274 (55.0)	350 (70.0)	463 (75.8)	83 (21.1%)	95 (15.6%)	88 (16.0%)	21 (7.3%)	4	9 (4.5%)
Postsynapsis (spireme)	0	75 (15.0)	2	311 (78.3)	513 (84.0)	452 (82.2)	235 (82.1)	148 (67.0%)	152 (76.0)
Prophase (chromosome formation) ..	1	0	0	0	1	7	23 (8.0)	31 (14.0)	27 (13.5)
First spindle ..	0	0	0	0	1	1	7	18	8
Daughter-cell stage	0	0	0	0	0	0	0	11	3
Megaspore stage.	0	0	0	0	0	0	0	9	0
Total number of ovules examined ...	498	500	611	394	610	550	286	221	199

From the above we may infer that the differentiation into the heterotype and homoeotype mitoses takes place at a stage later than synapsis. An exact stage of differentiation can be determined from the following observations:

1. The distinct chromosomes, appearing while the nuclear membrane

and nucleolus still persist, are invariably 16 (seldom 18). They are in peripheral arrangement (Figs. 21, 23, 24) and take on a piriform shape.

2. With or after the disappearance of the nuclear membrane and nucleolus the chromosomes retreat from the periphery towards the centre of the nuclear cavity. It is first at the period when the aggregated chromosomes are arranged in an equatorial plate, that we can find distinctly 16 chromosomes in some (Figs. 41-43) and chromosomes in other nuclei (Figs. 28, 36, 38). Each chromosome is in the former case oval, rod-shaped, or piriform, and in the latter it is as a rule a curved rod.

3. The 8- and 16-chromosomic spindles present a somewhat clear difference in width. The 8-chromosomic spindle is generally narrower than the 16-chromosomic one (compare Figs. 40, 45 and Figs. 43, 47, 48). This does not seem to be connected, as might be expected, with the spatial relation in the arrangement of the larger and smaller number of chromosomes. I take it rather as being due to the phase of arrangement of chromosomes in both cases. For, in the majority of cases, 8 chromosomes are found more closely together, often touching each other (Fig. 38), than 16 chromosomes aggregate (Fig. 41). In the former it frequently happens that a close approximation of chromosomes obscures their individuality, while we recognize in the latter case a certain space among chromosomes.

These observational facts demonstrate that the individual chromosomes may sometimes fuse pairwise on coming to the equatorial plate. Each chromosome on the 8-chromosomic spindle is, therefore, of bivalent nature. No other interpretation is possible for an apparent reduction of the chromosome number first at the metaphase stage. With this conception we can arrange with ease the several stages acquired by different nuclei about this period in their natural sequence. After retreating from the periphery 16 chromosomes come into the centre of the nuclear cavity, being drawn together (Fig. 25), loosely in some nuclei and very closely in others, sometimes so close as if to fuse altogether into an irregular mass. In some nuclei where the chromosomes are closely arranged, but in such a state as easily and distinctly countable, we can ascertain the decrease of the chromosome number, accompanying the appearance of larger, dumb-bell- or curved rod-shaped chromosomes. Such a phase (Figs. 26, 27, 28) shows beyond doubt the fusing process going on.

As it takes place previous to the appearance of the typical spindle, it cannot be mistaken as showing the splitting process of 8 bivalent chromosomes into daughter halves, the opposite process, taken at the late metaphase.

Such are our conclusions reached on the basis of apparent facts, which permit no speculative consideration on events of the nuclear features so far concerned. Attaching a great weight to these facts, we may now turn back upon the complicated process through which the chromosome is formed.

As already described in the first part of this paper, fine spireme threads in nearly homogeneous thickness throughout loosen out of the synaptic knot. They show first entanglement, but disperse gradually singly and traverse the entire cell cavity. Later, they arrange themselves regularly along the inner side of the nuclear membrane. The surface view at this stage reveals that they are apparently endlessly distributing themselves uniformly without frequent crossing (Fig. 7). While perhaps showing an increase in length, they become thinner in thickness but denser in arrangement. At this stage the course of the threads becomes irregular, making anastomotic connections at several points, often producing a network with somewhat regular meshes (Fig. 8). We often recognize two threads running closely parallel for greater or lesser distances (Figs. 9, 10). This seems to be an occasional event, since in no case have I been able to trace the parallel arrangement throughout their entire length. As far as my observation goes, there is no strong evidence for the general occurrence of a longitudinal split of the spireme or of the parallel association of two independent systems of threads (maternal and paternal), as interpreted in a vast majority of cases in reduction division.

At the time of an anastomotic arrangement, the spireme threads lose their homogeneity; sometimes thinner and thicker portions are distinguished, sometimes beaded accumulation of chromatic substance can be observed. Later, the meshes become irregular (Figs. 9, 11) and the threads become more dense at the angles of meshes. Such a change indicates the condensation process of the chromatic substance. It proceeds further to condense the substance at certain portions of the meshes, by which the network appears to be broken into a definite number of pieces of irregularly contracted meshes (Figs. 11-13). A certain figure (Fig. 12) representing a stage of this conduct may

sometimes resemble closely that given at the presynaptic reticulum (Fig. 1). But that it shows actually a postsynaptic stage can easily be confirmed by the seriation of the stages preceding and succeeding it.

Each chromatic mass thus formed represents the origin of an individual chromosome (Fig. 14). At first it shows no regularity in form, as the process of condensation presents a certain variation. Sometimes the chromatic substance appears to accumulate along two parallel segments of threads on the meshes (Figs. 14, 15), or throughout their peripheral segments in a loop or ring form (Fig. 17-19). Perhaps according to different manners of condensation the chromosome origin is represented as two parallel segments, a ring, or a loop, always with an irregular margin and joined to each other by linin threads. As a rule, there appear 16 of such chromosome origins. During the subsequent stages, in which further condensation and contraction ensue, it is observed that they assume an alveolated structure (Fig. 16), being presented perhaps by approximation or fusion of the two parallel segments, of the two arms of the loop, or of both sides of the ring. As the result, the chromosomes take on irregular broad bands (Fig. 22). Attaining to the final stage of condensation they assume a smooth, compact, and piriform appearance (Fig. 23).

The general features presented by the postsynaptic spireme, from its network arrangement till the formation of the chromosome segments, may show a striking resemblance to the corresponding stages of the reduction division in certain plants. For instance, in *Primula*-hybrids studied by DIGBY ('12), anastomosis can be observed in the postsynaptic spireme shortly before the loop formation. So that, in *Gastrodia* the frequent occurrence of the chromosome origin as a loop or ring may be with strong reason looked upon as the process of formation of the bivalent chromosome. In fact, certain figures are strongly suggestive of the reduction division in usual fashion; in well-differentiated sections two deeply staining masses are found in the matrix of the loops or rings, 16 pairs in full number (Figs. 19, 20). As showing a succeeding stage, the chromosomes reveal the duplex nature and exhibit precisely the same figure as the typical diakinesis occurring during the usual heterotype mitosis (Fig. 21). However, that they are not actually bivalent in nature, is evidenced by the fact that the number of such chromo-

somes is generally 16, just corresponding to the full set of univalent chromosomes.

The duplex character of the chromosome is a vexed question with me. We recall here the double type of chromosomes known in increasing instances of the somatic division, where it has been caused as the result of a longitudinal fission (see a review of GATES, '11). An occasional appearance or disappearance of this character may be perhaps due to an artifact, as LUNDEGARDH ('12) maintains.

It frequently occurs that 16 chromosomes thus formed are not all equal-sized or even equal-shaped. We find one or two longer ones in a rod form. First, the chromosomes arrange themselves in the periphery along the membrane (Figs. 21, 23, 24). The achromatic substance begins to organize into more or less fibrous structure (Fig. 24). When the chromosomes are retreating from the periphery, a multipolar spindle can be found at times (Fig. 25).

Among the chromosomes in the peripheral arrangement it is quite impossible to recognize their mutual connection. Notwithstanding this, we can conceive a certain affinity between each two of them, as evidenced by the act of the pairwise conjugation in coming to aggregate in the centre of the nuclear cavity. However, as already mentioned, the conjugation does not take place in all cases. When it occurs, we obtain 8 bivalent chromosomes on the equatorial plate; if not, 16 univalent chromosomes can be distinctly found. The mitotic process diverges first at this stage either to follow the heterotype or homoeotype division.

Nothing definite can be stated as to how the conjugation of the chromosomes can be effected in one case and omitted in the other. However, from the mechanism concerned, one may conceive that after the formation of the univalent chromosomes several processes involved in the ensuing mitotic stages follow in unco-ordinated rapidity; while the chromosomes are arranging on the equatorial plate, the other processes, such as the spindle formation, advance so far as to make the chromosomes risk the chance of conjugation and hasten to divide immediately into the daughter halves. If the relation is reversed, the chromosomes can approach each other so closely as to ensure conjugation. I often observed that the chromosomes became more closely

massed and came in contact with each other, as if fusion might take place among more than two, while the spindle was not yet formed. It is clear that the formation of the bivalent chromosome can succeed only when the chromosomes come to close approximation. To arrange closely or loosely is a slight matter, but it has a great significance in the present case upon the mode of the chromosome reduction.

As to the mode of the chromosome reduction, the serial stages we have so far studied, leave no room for any other explanation than we have attempted. If this be true, the reduction process is so peculiar that we cannot by any means come to harmonize it with any of the current views on this subject. The most peculiar point can be seen in the behaviour of the spireme developed through the synapsis stage, which, instead of being shortened and thickened, shows a tendency to become thinner and longer, to arrange itself in an anastomotic condition as seen in presynapsis. The process with which this spireme system is transformed into a definite number of chromosomes appears also as unique. Broadly speaking, however, the condensation phenomena of the prophase concerned with the development of chromosomes may find a certain accordance with those in the somatic mitosis which STRASBURGER accepts in the recent edition of his "Lehrbuch der Botanik" (1911). As far as the formation of the bivalent chromosome from the univalent is concerned, *Gastrodia* agrees in essential points with several *Oenothera*-species. In the pollen mother-cell of *O. rubrinervis* (GATES, '10), *O. Lamarckiana* (GEERTS, '09; DAVIS, '11), *O. biennis* (DAVIS, '10), and *O. gigas* (DAVIS, '11) the postsynaptic spireme derived, following the usual fashion, is cut off, during, before, or after the "second contraction," into the somatic number of chromosomes. In the spindle "there appears to be no system in the grouping of the chromosomes, as they are brought to the equatorial plate just previous to the metaphase of the mitosis" (DAVIS, '11, p. 949), and their generally scattered arrangement makes it impossible to determine whether alternate (adjacent) chromosomes on the spireme are always grouped in proper pairs which appear in the typical diakinesis. Notwithstanding this, the chromosomes grouped at the equatorial plate behave in the following stages so as to ensure the reduction of the number of chromosomes entering the daughter nuclei. Thus *Gastrodia* and *Oenothera* are in agreement for emphasizing the fact that in

the reduction process a certain significance placed upon telosynapsis and parasynapsis must be precluded.

In the subsequent nuclear features the behaviour of the chromosomes presents a slight difference in certain points, according to whether it is bivalent or univalent. The bivalent chromosome is at first long (Figs. 28, 36), but at the time the spindle figure is formed it contracts more or less, still being longer than the univalent chromosome (Fig. 38). As both sides of the bivalent chromosome are so intimately associated as to display no sign of a duplex nature, it is difficult to learn at what plane a split takes place during heterotype division. My slides only show that at the metaphase the bivalent chromosome increases its breadth, appearing, as it were, to split longitudinally. In side view it is very often found as assuming a rhomboid form (Figs. 39, 40). Unfortunately, a clear figure giving the mutual relation of the freshly split daughter chromosomes was not obtainable. The latter departing from the equatorial plate towards both poles are, as a rule, slightly curved rods (Fig. 46), being more slender than the mother. Shorter chromosomes seldom occur (Fig. 45). During the subsequent nuclear generations the chromosome retains its typical form (see Figs. 59, 60, 74, 81, 82, 87).

In the case where 16 univalent chromosomes form an equatorial plate each chromosome assumes generally an oval form, and in separating into daughter halves it takes on a dumb-bell shape throughout all nuclear generations (Figs. 44, 62, 73). The daughter chromosomes are also oval, being far shorter than those derived from the bivalent chromosome (Figs. 47, 48). However, in succeeding mitoses they are often represented as a short rod (Figs. 63, 65, 66).

The mitosis in the somatic cells has been studied on the ovarian tissue. Broadly speaking, it follows the usual type; the reticulum is converted into homogeneous spireme threads which, being apparently continuous, run freely along the peripheral portion of the nuclear cavity (Fig. 29). They are segmented directly into the diploid number of the chromosomes, being first connected with each other by delicate linin fibres (Fig. 30). Each segment shortens and thickens, and is fashioned into a compact chromosome in a long rod form (Fig. 31). At the prophase no fission is observed at all. The length and breadth of the chromosome present a certain variation. A careful

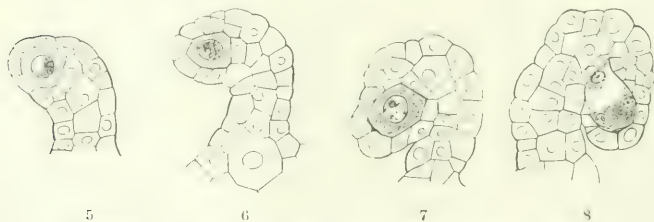
counting gives 16 univalent chromosomes (Figs. 32-35). Thus, the mitosis in the somatic and archesporium cell begins with a different process, but in the latter it may bring forward the same result as in the former.

Similar to the haploid megaspore, the diploid one is formed through the tetrad divisions. This is evidenced by the fact that two crescent cups, showing the two obliterated cells derived by the preceding two divisions, are present over the megaspore in which the nuclear division goes on to distribute 16 chromosomes on each pole of the spindle (Fig. 73).

6. SPOROPHYTIC PARTS IN RELATION TO THE GAMETOPHYTE.

The growth of the ovary, the development of the sporophytic and gametophytic parts of the ovule proceed pace by pace before, as well as after fertilization. It is evident that they are in a most intimate relation to each other. The experiment, carried out with a view to analyse their mutual relation, makes it desirable to record here the developmental phases of the sporophytic parts of the ovule under ordinary conditions.

In a flower-bud which appears to open after 2 days, the ovule develops as a short cylindrical process, consisting of a central row of cells surrounded by an epidermal cell-layer. The apex of the process is slightly curved. The archesporium cell is already differentiated from the other cells of the central row, and on account of its greater size the apex of the ovule is slightly swollen. The nucleus enters mostly midsynapsis, but is yet in part at presynapsis (Table II).



Text-fig. 5. An ovule a day before bloom (microtome section). $\times 400$.

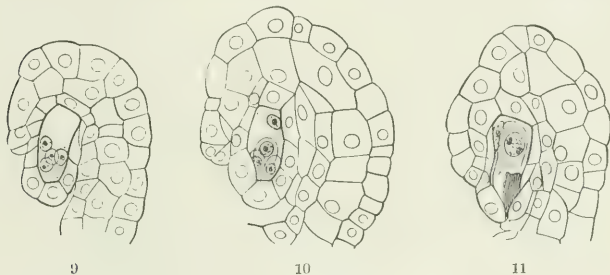
Text-fig. 6. The same the day of bloom (microtome section). $\times 400$.

Text-fig. 7. The same a day after bloom (microtome section). $\times 400$.

Text-fig. 8. The same 2 days after bloom (microtome section). $\times 400$.

On the day preceding bloom the ovary shows a slight growth. The curvature of the ovule becomes more conspicuous (Text-fig. 5); at its concave side one or two small cells begin to develop, being cut off from the epidermal cell. Most archesporium nuclei have passed through the presynapsis stage and some are at midsynapsis, while in the majority the postsynapsis is attained; this is shown by the loosening of the thread from the knot.

On the day of bloom the ovary shows no marked further growth; the ovule is directed transversally (Text-fig. 6). The archesporium nucleus advances mostly to the postsynapsis stage. Only a few are at the beginning of



Text-fig. 9. An ovule 3 days after bloom (microtome section). $\times 400$.

Text-fig. 10. The same 4 days after bloom (microtome section). $\times 400$.

Text-fig. 11. The same 4 day after bloom; fertilization takes place (microtome section). $\times 400$.

loosening the thread from the knot, and some are developing chromosomes, while the spindle is rarely already formed (compare Table II).

PACE ('07) describes in *Cypripedium*: "In flowers just opening the spireme of the mother cell is found, and the synapsis stage is usually reached by the time the flower is in full bloom." Here the ovular development is slightly slower than in *Gastrodia*.

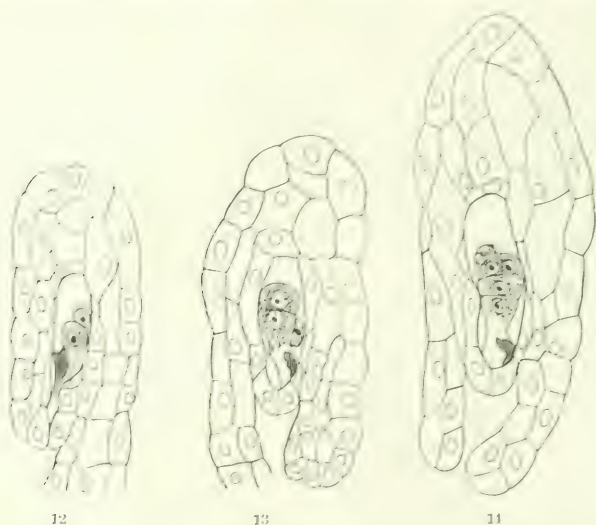
Pollination has been made on the flowering day and the subsequent stage was observed on successive days.

After a day the ovule is directed obliquely downwards, and just at the level of the basal portion of the archesporium the surface of the ovule shows a slight swelling at the concave side. The swelling is due to the appearance of the rudiment of the integument, being caused by the cell multiplication beneath the epidermis (Text-fig. 7). At this period the archesporium cells in

the same ovary present a wider range of the nuclear feature than before. In most ovaries we recognize their first or second division, leading in the latter case to the formation of megaspores.

After 2 days the ovule becomes quite anatropous and the megaspore undergoes one or two divisions, thus producing the embryo-sac at the two- or four-nucleate stage (Text-fig. 8).

After 3 days a single integument becomes somewhat conspicuous, sometimes as a short process reaching half the way of the elongated embryo-sac.



Text-fig. 12. An ovule 5 days after bloom (microtome section). $\times 400$.

Text-fig. 13. The same 6 days after bloom (microtome section). $\times 400$.

Text-fig. 14. The same 7 days after bloom (microtome section). $\times 400$.

At this period the four nuclei of the sac occupy their final position (Text-fig. 9).

Up to this period we observe, though in an exceedingly slight degree, a continuous enlargement of the ovary. Throughout this interval the ovary assumes a triangular form, with three edges diverging towards the top where the perigone rests. The ovule has also made the corresponding growth.

After 4 days the ovule enlarges more markedly (Text-fig. 10). The nucellus, integument, and funiculus become clearly differentiated. The nucellus consists of a single cell layer encasing the embryo-sac, and its lower half is surrounded by the integument or funiculus tissue. The development of the integument is still incomplete; it consists of two layers of cells, often three layers at the marginal portion.

Usually fertilization takes place on this day. The pollen-tube penetrates into the sac through the intercellular space surrounded usually by four nucellular cells (Text-fig. 11). The latter cells are turgescient, rich in plasmic contents, and larger than those at other portions of the nucellus, which are at this period somewhat compressed between the overlying tissue and the enlarging sac.

What is worthy of mentioning in the external appearance of the ovary after fertilization is its rapid increase in size (see Text-fig. 15). Previous to fertilization it has enlarged with retention of its proper form. Now it commences to elongate much more than to thicken. This sudden change in the developmental feature of the ovary may be taken as an effect of fertilization. Likewise, a sudden change occurs in the just fertilized ovule, being manifested by its longitudinal elongation, owing in part to a renewed marginal growth of the integument (compare Text-figs. 11 and 12).

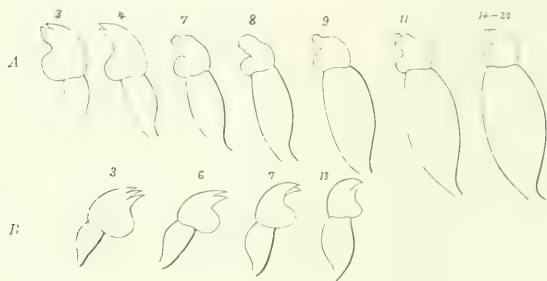
After 5 days the fertilized egg begins to divide, the two-celled stage being attained. At the same time remarkable developmental changes take place in all the sporophytic tissues of the ovule. The embryo-sac and almost all the ovular cells are enlarged, leading to the growth of the ovule itself especially towards its longitudinal direction, and meanwhile the integument grows up to or over the level of the nucellus (Text-fig. 12) by a rapid cell-division on the margin. Associated with this sudden increase of activity in the ovule the ovary is promoted in growth.

Hereafter the daily growth of the ovary and ovule is exceedingly remarkable. In the ovule all the enlarging cells elongate more and more, whereupon its chalazal end tends to taper. At the micropylar end the multiplication of cells in the integument, which leads its free portion to protrude far beyond the nucellus as a tissue composed of three cell-layers, brings about also a similar tapering, being enhanced by an elongation of the composing

cells. The mature seed thus assumes a spindle shape (compare Text-figs. 13, 14, and 27).

Except on the margin of the integument no cell-division seems to take place in the ovular tissue after fertilization, so that a conspicuous enlargement of the seed-forming ovule is mainly due to the growth of the component cells themselves. During the seed formation the embryo-sac is surrounded from the beginning to the last by four cell-layers, including a compressed cell-layer of the nucellus, which often becomes quite indistinct.

The subepidermal cells of the ovule are more enlarged than the epidermal ones, becoming remarkably devoid of the protoplast. As an exception, we



Text-fig. 15. Development of two ovaries on the same inflorescence axis. *A*, pollinated with the *Gastrodia*-pollinium; *B*, unpollinated. Nat. size¹.

find a cell just at the base of the sac, without undergoing any essential change; it retains nearly the original form and size, containing the denser protoplast. The nucellar cells at the micropylar end, having free outer surface, are at the same state, being turgescient and rich in granular plasmic contents and having the nuclei apparently at an active condition (Fig. 114). These characters can be maintained as long as the seed comes nearly to maturity. Their significance will be considered later on.

Up to the time the seed attains a final form and size, which it will arrive at, in most cases, about 12-13 days after bloom, the epidermal cells remain in a turgescient state, accumulating large grains of starch. Now, they come

1. In this and the following similar figures Arabic numerals show the number of days after bloom.

to differentiate into the testa, by producing a network relief on the wall. In this process the outer free wall remains thin and bulges outwards due to strong turgor of the cell, but throughout the other portions of the wall a reticulate thickening takes place (Text-fig. 27). When the thickening is completed, starch disappears entirely from the cavity, and by the loss of turgor the outer expanded wall shrinks and breaks ultimately. This process accords in essential points with that taken during the formation of the seed-coat in *Rafflesia*, studied by ERNST and SCHMID ('13) conjointly. The completion of the testa takes place in 14-15 days after bloom, with which the seed can be said to attain maturity. At the same time the ovary attains the maximal size (Text-fig. 15).

Thus, the ovular structure of *Gastrodia* may be considered as one of the simplest among the Orchidaceae; it is especially remarkable that the development of a single integument is much deferred; it is yet rudimental at the time of fertilization and its rapid development is induced by the act of fertilization.

During the development of the ovary into the capsule no essential histological change occurs. The growth of the ovary takes place simply by the corresponding enlargement of the component cells, without their multiplication at all. This is clearly shown by comparing the structure of the wall of a young ovary and that of an adult capsule.

The dehiscence of the capsule takes place some days after it has attained the maximal growth. The interval is variable, depending on the vigour of the plant. It is usual that when the fruit approaches maturity, the inflorescence axis is thrown into an unhealthy condition, producing decayed spots attacked by mould fungi. It hastens the drying of the capsule. When such a condition arrives early, the capsule dehisces about 15-16 days after bloom. However, when the axis is healthy, the dehiscence is delayed until about 23 days after bloom.

IV. Discussion of the Results.

1. NUTRITION OF THE EMBRYO.

In almost all orchids the endosperm nucleus does not multiply. Though in exceptional cases it divides once or twice (*Cypripedium*), there is no

formation of endosperm, as the resulting nuclei degenerate sooner or later. On this account, the poorly developed embryo of the Orchidaceae, differing from that of the typical Angiosperms, must draw its nutritive substances directly from the sporophytic portions. As recapitulated by COULTER and CHAMBERLAIN ('03, p. 194), the haustorium-like development of suspensors in most orchids must be looked upon as having significance upon the direct nutrition of the embryo by the suspensors. STRASBURGER (Bot. Practicum, '97, p. 580) describes the embryo of *Orchis pallens* as having a cuticular layer on its surface, but not on the suspensor. This points out impossibility of passage of nutritive supplies from the sac to the embryo. The suspensor of *Gastrodia* is of the usual structure, and agreeing with COULTER and CHAMBERLAIN'S statement, "Every suspensor is an haustorium" (p. 113), I can demonstrate with high probability its nutritive function, supported by the nature of the tissue to which the suspensor is in direct connection.

The apical nucellar cells—generally two in longitudinal section of the ovule—to which the suspensor attaches, have the outer wall freely exposed. They take the position as a connecting link between the suspensor and the integument, and retain for long a turgescient state, with the conspicuous nuclei and dense plasm, showing in general aspect a high activity. They function probably as an absorbing organ of food materials from the funiculus, to supply these to the growing embryo. At the time of the completion of the embryo-sac, the innermost nucellar cells directly surrounding it become stretched and compressed, more pronounced at later stages, so that it appears impossible for them to maintain their conductive activity for food materials from the outside into the sac.

As to the significance of the single basal nucellar cell (Fig. 114) at the charazal end, which shows quite the same character as the cells at the micropylar end, it may be likewise considered as conveyance for the food materials to the sac. As the sac does not develop the endosperm, it demands no active accumulation of the food materials, but as its endosperm nucleus and cytoplasm ought to maintain their activity until the embryo attains a definite size, they must require a nutritive connection with outside. The necessity of supply of substances will be seen from the osmotic pressure of the sap in the sac. The inner wall of the embryo-sac and the

surface of the multicellular embryo do not show the presence of cuticle, pointing to the possibility of nutritive communication through these places. But from what has been considered above about the ovular tissues, I am inclined to think that the embryo and the embryo-sac obtain nutritive supplies independent of each other through the specially developed cells.

The distribution of starch-grains in the fertilized ovule may be of some interest. In the ovule in which the embryo attains a few-celled stage, the starch-grains accumulate most pronouncedly at the charazal end. They are there as large as in cells of the placenta, where the starch is being drawn in from the store house. Towards the micropylar end a decrease in amount and size of starch-grains is observed. Such an un-uniform distribution of starch may be explained as being connected with different natures of activity of the cells at both ends of the ovule. At the charazal end it may be consumed for enlarging the cells, while at the micropylar end it is chiefly wasted in multiplying the integumental cells. In contrast with all these cells, both the micropylar and charazal ones of the nucellus, mentioned above, contain an exceedingly small amount of fine starch-grains. A similar amount of starch is visible in the embryo-cells, but not in the protoplast of the embryo-sac.

2. RAPIDITY OF THE SEED MATURATION.

As already described, fertilization takes place under ordinary conditions 4 days after pollination and the two-celled proembryo appears on the next day. Hereafter, the development proceeds very rapidly and within 2 weeks from the day of bloom the seed nearly attains maturity. Such a rapid development seems to be exceptional in the Orchidaceae. For, of numerous species hitherto investigated, most tropical ones are fertilized, according to GUIGNARD ('86) and HILDEBRAND (see GUIGNARD's paper), in 1-6 months after pollination, while the endemic (European) species effect fertilization in 2-6 weeks¹. *Listera ovata* (GUIGNARD, p. 224) and *Neottia nidus-avis* (GUIGNARD, p. 224) are said to produce a rudimental embryo after 10 days of pollination, showing the most rapid development hitherto known.

1. COULTER and CHAMBERLAIN (p. 147) quote HOFMEISTER's work in which the maturation period is given as from 10 days to several months among the orchids.

From an ecological point of view PRITZER ('89, p. 73) remarks as to the period of the fruit maturation, that in the tropics the orchids, which flower and are pollinated in the rainy period, mature the seed so as to disseminate it in the next rainy period, spending the interposed dry season for its maturing period. I would like to consider *Gastrodia's* so rapid seed formation as bearing upon an unique mode of nutrition. In this orchid the reserve material in the flowering tuber is utilized in greater part for the formation of, a long inflorescence axis, which often attains more than one metre in length and bears numerous flowers, exceeding sometimes 60 in number in a larger tuber. During the vegetative period the reserve material must also be wasted in part for respiration, the loss being greater the longer the period. The rapid development of the seed may be a contrivance for economizing the material. At the time when the fruit comes nearly to maturity, the tuber, which until then has been solid, being packed with starch, becomes hollow and somewhat translucent, showing exhaustion. Unlike other orchids the flowering individual of *Gastrodia* is unable to take up any additional nutritive material from the surrounding medium, so that it may be admitted on theoretical grounds that the length of the maturation period is commensurate to the limited amount of food materials stored up in the tuber.

3. SIGNIFICANCE OF THE REDUCED EMBRYO-SAC.

Since CHODAT and BERNARD ('00) first described in *Helosis* (Balanophoraceae) the 4-nucleate embryo-sac, such an atypical structure of the sac has become known among various families, thus in *Limncharis* (Butomaceae) (HALL, '02), *Cypripedium* (Orchidaceae) (PACE, '07), several Onagraceae (MODILEWSKI, '09; GEERTS '09; WERNER, '14), the Podostemaceae (WENT, '09; MAGNUS, '13), *Clintonia* (Campanulaceae) (SMITH, '11), and *Garcinia* (Rubiaceae) (TREUB, '11). The morphological nature of the sac is not everywhere alike in these instances. In the Onagraceae, *Helosis*, *Clintonia*, and *Garcinia* the sac is derived from one megaspore, while in *Limncharis* (?), *Cypripedium*, and the Podostemaceae two megaspores take part in forming the sac. SHARP ('12) reported in *Bletia*, which possesses normally the 8-nucleate sac, an abnormal one with 4 or 6 nuclei, being derived from one or four megaspores. The same thing has recently become known in *Gyrostachys* (PACE, '14). In either

case the presence of 4 nuclei in a complete embryo-sac points to the fact, that the number of divisions which intervene between the mother-cell and the complete sac are reduced. The frequency of division of the megaspores may vary according to the number entering the sac; only one division is sufficient when two megaspores occur, while two divisions are required when one megaspore occurs. Somewhat different is the nuclear division in *Helosis*, *Limncharis*, and some of the Podostemaceae (*Lavia* and *Lacideae*); at the two-nucleate stage of the sac the lower antipodal nucleus degenerates and the upper micropylar one is concerned with the formation of the sac, by undergoing two divisions.

Thus the reduced form of the embryo-sac presents diverse types from the morphological point of view, and as to their significance it appears impossible to present a general view.

As already announced by several authors, Angiosperms which possess typically the 8-nucleate gametophyte are derived from an ancestor possessing a larger number of gametophytic cells. From this point of view it may be theoretically assumed that the gametophyte so much reduced as in the plants mentioned above would indicate an advanced form at the progressive line of evolution in Angiosperms (see MAGNUS, '13, p. 331).

With this statement we must not be too hasty to conceive the phylogenetic position of plants possessing such a reduced embryo-sac, for there remains a point to be considered, which concerns the correlation between the vegetative and generative spheres. This may be better shown by the Podostemaceae, where the reduced embryo-sac is universal and the vegetative sphere exhibits a uniformity. This family possesses a highly reduced vegetative organ which, however, is well adapted to vegetation in a shallow stream (WILLIS, '02). According to WENT ('08) and W. MAGNUS ('13), under the extreme heat of the tropical sun, the plant body becomes suddenly exposed to a great drought; then the flower opens and finishes fertilization to mature the seed with great rapidity, but under a too limited nutrition. The direct nutritive connection of the embryo with the funiculus, not with the endosperm as in most Angiosperms, is related in some way to the reduction of the antipodal apparatus (including the lower pole nucleus). MAGNUS looks upon the reduced gametophyte and vegetative organ as showing a certain pro-

gression in the evolution of Angiosperms, stating that the Podostemaceae are, not retrograde, but progressed forms, "die unter extremen Lebensbedingungen neben weitgehenden Anpassungen der vegetativen Sphäre einen Anstoss zur Fortentwicklung der generativen Sphäre erfahren haben, die weit über das normale Mass der Angiospermen hinausgeht" (p. 331).

On account of the highly specialised characters of the vegetative and reproductive organs the Orchidaceae may be regarded as a phylogenetically advanced plant group. This view is strengthened by a reduction of the gametophyte throughout the family. The reduction is manifested by the suppression of the endosperm formation, leading the embryo to depend for its nutrition on the sporophytic tissue. In such a specialised gametophyte the antipodal cells and the pole nuclei, though they occur in fact in most orchids, may be superfluous structures. It is, therefore, not strange that such a physiological adaptation in the gametophyte may bring forth a morphological adaptation, that is, the development of the 4-nucleate embryo-sac as a more progressed form than the typical 8-nucleate one. For the explanation of the occurrence of such a reduced embryo-sac in *Gastrodia* I must emphasize the fact, that this orchid shows a striking ecological resemblance with the Podostemaceae; for the reproductive organ develops at the expense of the food stored in the tuber alone, and, as already discussed, the need of economizing it is pressing. A great rapidity in the development of the flower shoot, the gametophyte, and the fruit, in addition to extreme simplicity in the embryo and seed, which is universal in the Orchidaceae, may be considered an important character in the generative sphere, acquired in correlation with such a nutritive condition. Extending this view further, a reduction in the embryo-sac is a more requisite adaptation, since the suppression of the unserviceable antipodal apparatus can simplify the process and hasten the progress of the gametophytic development. Thus in agreement with MAGNUS' statement on the Podostemaceae, we can scarcely be wrong in concluding, that the 4-nucleate embryo-sac of *Gastrodia* represents a form of the gametophyte of the Orchidaceae more specialised than the type, being influenced in correlation, to a certain extent, with the highly characteristic vegetative sphere.

4. CHROMOSOME BEHAVIOUR AS TO THE EVOLUTION OF PARTHENOGENESIS.

The behaviour of chromosomes associated with the reduction division is very peculiar in *Gastrodia*. Although this plant is habitually amphimictic and any experimental means fails to induce the parthenogenetic development of seed, the cytological evidences seem to justify us to assume the probability of parthenogenesis. The chief interest in this connection lies in the point that parthenogenesis is developed from amphimixis, with certain modifications of cytological processes by which the egg-cell is produced.

As far as reported, the first mitosis in the embryo-sac mother-cell of parthenogenetic plants begins with the usual heterotypic process, until synapsis is attained. As, however, univalent chromosomes are derived, we must search for the first impetus to the parthenogenetic development among stages comprised in postsynapsis.

In reduction division the mode of the formation of a bivalent chromosome was diligently studied and widely discussed by many competent cytologists. In parthenogenesis the process with which a univalent chromosome is formed, instead of a bivalent, offers a subject of study in close association with this mode of division. But as to how the univalent chromosome is organized in the postsynapsis stage, and what difference we can recognize in the behaviour of the postsynaptic spireme in deriving the univalent chromosome in parthenogenetic plants and the bivalent one in amphimictic plants, both comprised in one and the same genus, we have not yet a detailed account in harmony with our present knowledge of reduction division.

In his study on the parthenogenetic *Antennaria alpina*, JUEL ('00) has first called attention to the first mitosis in the embryo-sac mother-cell in this respect. He found the synapsis stage, but the spireme derived presented the form and distribution which characterized the resting stage of the nucleus. While in the amphimictic *A. dioica* synapsis was followed by the typical diakinesis, the postsynaptic spireme in *A. alpina* was segmented, following the manner as observed in the somatic cell. Seriation of stages involved in synapsis and postsynapsis is not sufficiently complete to make a precise cytological distinction between both species of *Antennaria*. A comparative study on the postsynapsis stage has recently been made by OSAWA ('13) on

the amphimictic and parthenogenetic *Turaxacum*-species. According to him, while synapsis unravels the parallel spiremes in the amphimictic *T. platycarpum*, only a single thread arises in the parthenogenetic *T. albidum*. In the latter species the transverse segmentation of the thickened and condensed spireme thread gives rise to the full number of univalent chromosomes. In the parthenogenetic *T. officinale* JUEL ('04, '05) has already ascertained the same thing.

According to the current views on the chromosome reduction, both the parasynapsis (side-by-side pairing) and telosynapsis (end-to-end pairing) methods are equally accepted in deriving a bivalent chromosome. From this fact it seems reasonable to infer that in *Turaxacum* the amphimictic species derives a bivalent chromosome by a side-by-side pairing of univalent chromosomes, while the parthenogenetic species forms a univalent chromosome by a slight modification of the telosynapsis process, the modification being involved in the segmentation of the spireme into the full set of the somatic number, instead of the paired set which is to be secured, as some authors insist, by the "second contraction." Thus we may conceive a fundamental difference among *Turaxacum*-species as regards the orientation of chromosomes in the embryo-sac mother-cell.

On coming upon those parthenogenetic plants which develop both diploid and haploid eggs, the behaviour of chromosomes offers a more complicated subject for consideration. *Thalictrum purpurascens* (OVERTON, '02, '04), *Hieracium excellens* (ROSENBERG, '07), *Houttuynia cordata* (SHIBATA and MIYAKE, '08), and perhaps *Burmannia coelestis* (ERNST and BERNARD, '12) afford the examples, but no precise account regarding the process of the simultaneous development of both bivalent and univalent chromosomes from the synaptic spireme has hitherto been given. That in one and the same ovary some embryo-sac mother-cells develop the parallel spiremes and others a single univalent spireme in the postsynapsis stage might perhaps be possible, but it appears to me rather inconceivable on account of the fundamental difference in the arrangement of the spireme. If we agree with ROSENBERG's ('07) statement that the parallel spiremes occur in *Hieracium* in the postsynapsis stage of both pollen and embryo-sac mother-cells, then we are at a loss to understand how the omission of reduction division is effected in some mother-

cells. It may be remarked that BEER ('12) advocates telosynapsis in his study on the pollen development in several species of the Compositae; this elucidates the explanation upon the point under discussion.

The events in the postsynapsis stage in the parthenogenetic plants are interesting from several points of view. If the occurrence of the parallel spiremes (paternal and maternal) be universal, the parthenogenetic mitosis would be differentiated first at or after the diakinesis stage. If both parallel and single spiremes occur in one and the same plant, we may place the starting point for the parthenogenetic mitosis upon the synapsis or a previous stage. Thus the acceptance of the parasynaptic origin of chromosomes brings the matter in confusion. But, on the basis of the mechanism of telosynapsis, the process of the parthenogenetic mitosis may be explained more simply and clearly. The upholder of telosynapsis regards the postsynaptic parallel spiremes as caused by fission of a univalent spireme, instead of being the pairing of two spiremes, as parasynaptists maintain (see DIGBY, '10). MEVES ('08) has already expressed the opinion that the parallelism in parthenogenetic eggs is inexplicable, if it actually represents the pairing of homologous parental chromosomes. According to telosynapsis, only a slight change in the behaviour of the postsynaptic spireme, that is, the omission of the "second contraction" (DIGBY, '10), may turn reduction division to somatic mitosis.

Turning upon *Gastrodia*, a peculiar type of reduction division may be looked upon as adapted for ensuring the partial parthenogenetic development. Strictly speaking, the achievement of postsynapsis is not in accord with either parasynapsis or telosynapsis. The postsynaptic spireme is segmented in all cases, at the omission of the typical diakinesis stage, into the full number of somatic chromosomes. The criterion, in what way the heterotype mitosis is secured, lies at the stage of the formation of an equatorial plate. The formation of the bivalent chromosome is in this case deferred too far, and a slight change of internal or external conditions is liable to render it unsuccessful. As a whole, the behaviour of the chromosomes is here rather favourable for somatic mitosis, so that we possibly can regard such a type of the heterotype mitosis as occupying the lowest rank in the line of the cytological evolution of parthenogenesis. If we admit that parthenogenesis is developed

from amphimixis, such a mode of reduction division would perhaps have a phylogenetic connection with telosynapsis.

Although in *Gastrodia* parthenogenesis is warranted by the nuclear mechanism, other conditions do not harmonize to realise it, so that the formation of the embryo is secured only by the amphimictic development of the haploid egg. In this respect *Gastrodia* may be considered as representing an intermediate form between amphimictic and habitually parthenogenetic plants. In *Hieracium excellens*, *Houttuynia cordata*, *Burmannia coelestis*, etc. a greater or lesser number of haploid eggs are produced, but parthenogenesis advances so far as to produce diploid eggs in great majority, and as to hinder fertilization by producing sterile pollen-grains. *Thalictrum purpurascens*, producing fertile pollen-grains, is, however, capable of both amphimictic and parthenogenetic development in nearly equal degree. As a remarkable fact, *Hieracium excellens*, though its pollen-grains are sterile, can assume an amphimictic development by crossing with other species. From these facts we can conceive several stages in the parthenogenetic evolution, and can consider *Gastrodia*, *Thalictrum*, and *Hieracium* as representing a progressive series in the evolutionary line.

Further, a peculiar mode of reduction division in several *Oenothera*-species deserves consideration in relation to parthenogenetic evolution. As already mentioned, the mechanism of the chromosome reduction is here too unstable, just as in *Gastrodia*. The mode of the chromosome formation resembles rather that in the parthenogenetic *Taraxacum* studied by OSAWA ('13). It is interesting that *Taraxacum* performs habitually the somatic mitosis, while *Oenothera* is disposed to undergo the heterotype mitosis. However, if we keep in mind that the heterotype mitosis may occur occasionally in *Taraxacum*, as OSAWA remarks, we may conceive in *Oenothera* a similar state in reciprocal relation, that is, an occasional omission of the reduction division. In harmony with this view GATES ('07, '09) supposes on theoretical grounds that *Oenothera lata*, one of the mutants of *Lamarckiana*, is partly parthenogenetic. This supposition of GATES and a scattered arrangement of univalent chromosomes previous to the metaphase stage of the heterotype mitosis agree so well as to suggest, that a further investigation will prove parthenogenesis in some *Oenothera*-species to be a fact. Relating to this

matter, the occurrence of sterile ovules in several *Oenotheras* (GEERTS, '09) gives a further suggestion. In *Gastrodia* the sterility of some ovules actually occurs, being indeed beyond all doubt in connection with the instability of reduction division, which brings about the development of the diploid eggs. Although GEERTS' careful study on the tetrad divisions of the embryo-sac mother-cell leaves no doubt of heterotype mitosis ensuing upon both sterile and fertile ovules, the similarity of its reduction process with that of *Gastrodia* leads us to conjecture that the sterility may possibly be caused in part by the omission of the chromosome reduction. With this assumption *Oenothera* and *Gastrodia* are brought to an exact agreement in respect to the mode of reduction division, to the partial sterility of ovules, and to the four-nucleate nature of the embryo-sac. So that *Oenothera*, like *Gastrodia*, may be assumed as forming, in cytological features, a link in the evolutionary line of parthenogenesis.

The cytological facts we know at present regarding parthenogenesis are yet insufficient to formulate any hypothesis on the behaviour of chromosomes in the embryo-sac mother-cell. But from what has been considered, it is not quite inconceivable that there may exist intermediate forms in the evolution of parthenogenesis from amphimixis, which can be characterized by the behaviour of chromosomes. Further, I emphasize that the study on mitosis in the embryo-sac mother-cell in partially parthenogenetic plants, whatever results it might bring forth, would claim a participation in discussing the plan of the formation of chromosomes in heterotype mitosis, inasmuch as a diligent study of somatic mitosis will elucidate, whether the parallelism of the heterotypic and somatic prophase is homologous or not (see a critical review of LUNDEGARDH, '12).

V. Unpollinated Flowers under Different Conditions.

As may be seen from the foregoing description, the ovule is yet rudimental at the flowering stage. Being pollinated at that period, it can develop further in association with the elongation of the pollen-tube. Such an unusual delay of the ovular development is a wide-spread character in the

Orchidaceae, and it has been almost generally accepted that pollination initiates the completion of the embryo-sac. With a view to ascertain whether the same thing is true for *Gastrodia*, unpollinated flowers were subjected to normal and several other conditions, and their subsequent phases of development were followed on successive days, when necessary, on microtome sections.

1. THE FLOWER ON THE INFLORESCENCE AXIS BEARING FERTILIZED FLOWERS.

When many flowers in an inflorescence are fertilized and go to develop the fruit, the food material, contained in the entire stock, would be consumed, in greater part, by them. We presume that this condition may have a certain influence upon the nutrition and the postfloral development of the unpollinated flowers in the same inflorescence.

Experiment 1.

Several stocks were employed. The nutritive condition might be more or less variable among them. The relative number of the unpollinated to the pollinated flowers were not strictly equal in all the inflorescences, though the majority of flowers in each inflorescence was subjected to fruit formation by pollination. As the unpollinated flowers examined on successive days originated from different inflorescences, no warrant could be made as to the fact that the flowers examined had all been strictly at the same progress in their development.

1. In 1910 the material was studied in microtome sections.

After 2-3 days of bloom, no distinction could be seen between the pollinated and unpollinated flowers as regards the ovarian and ovular development.

After 4 days the pollinated flowers had been fertilized, but the embryo-sac of the unpollinated flowers was mostly at the tetranucleate stage and the egg-apparatus was not yet organized.

After 5 days the arrangement of the egg-apparatus was completed in some ovules, but often the pole nucleus showed obliteration, alone or together with the synergids, leaving the egg-cell alive and healthy. In many sacs, again, the plasm was massed at the upper portion, in which two nuclei were embedded, whereas the other two nuclei lay almost freely at the lower, charazal portion, sometimes being much smaller than the upper ones and sometimes being in degeneration (Fig. 85).

After 6-7 days the embryo-sac in almost all ovules showed shrinkage attended by the disorganization of the egg-apparatus. The ovular tissue attained in development a stage similar to the fertilization stage in the pollinated flower.

An early disorganization of the embryo-sac, or an unequal distribution of the plasm in the sac, could also be observed in the pollinated flower, but

it was far more striking in the present case. Perhaps it might be ascribed in part to an unfavourable nutritive condition of the stocks used.

2. In 1913, stocks apparently more vigorous than those used for the preceding experiment were selected.

After 4 days the perigone remained fresh; the ovary was slightly enlarged; and the egg-apparatus was completed in most ovules.

After 6 days the perigone wilted; in general form the ovule was similar to that of the pollinated flower at the 4-days stage. The embryo-sac with the vacuolate egg was healthy and the pole nucleus became larger, presenting a prominent reticulum. In some sacs the egg-apparatus did not come to final development, in others it was beginning to degenerate.

After 8 days the ovary showed a slight growth (Text-fig. 16); in almost all ovules the nucellus appeared more or less projected out; and the embryo-sac assumed a somewhat shrunken form, containing the intact egg-apparatus. In very few ovules the integument extended up to the level of the nucellus, and their size was increased, especially in breadth.

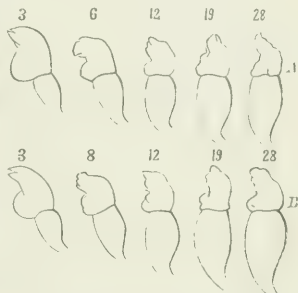
After 9 days the stigma was yet mucous; the ovule had the exposed nucellus and remained in size just as in the preceding day; the synergids were beginning to degenerate, while the egg-cell remained intact, becoming larger and swollen.

After 10-11 days the stigma yet retained a mucous consistence; the ovary grew further a little and on some stocks it acquired an erect position; the nucellus was exposed in many ovules which were in size and form as at the fertilization stage; in most ovules the egg-apparatus degenerated, while in a few it was quite healthy; the latter ovules showed a gigantic growth.¹

We recognized already after 8 days certain ovules attaining a marked growth, becoming more prominent after 10-11 days, mainly due to the enlargement of the composing cells and to the cell multiplication at the marginal portion of the integument. At this stage the growing ovules exhibited a wide range in size.

After 14 days the erect ovary became a little more enlarged, and the surviving ovules became more or less pulverous. The smaller ovule remained in size as at the fertilization stage, and the larger one became 3 times as large as that. The latter took on an oblong form, with the swollen epidermis containing large grains of starch.

After 23-24 days the ovary advanced in growth and developed into the fruit. In dissecting it, the pulverous embryoless seeds, arisen from the surviving ovules, easily detached from the placenta as observed in the normal,



Text-fig. 16. A, an unpollinated flower; B, a flower pollinated after 8 days of bloom (both from the same inflorescence). Nat. size.

1. "Gigantic growth" means a vigorous growth of the unpollinated ovule, just as the fertilized one.

fruit. The seed showed no essential difference in appearance from the normal, except in having rounded ends. At this stage the development of the testa was nearly completed.

The number of the seed derived from a single ovary was somewhat variable; in any case it was far less than that obtained from the fertilized flower. Again the mature seed presented a great variation in size. At the period of the seed maturation the fruit-wall ruptured along six longitudinal lines in quite the same manner as in the normal capsule. The period of dehiscence varied according to the healthy and unhealthy condition of the inflorescence axis; at the earliest, it took place after 23 days on the axis, giving symptoms of diseased condition; on a healthy axis it took place after 29 days of bloom. Drying ensued soon upon the dehiscent capsule.

Results:—The ovule, though rudimental at the time of bloom, continues, in case of the prevention of pollination, its further development parallel with that in the pollinated flower, and it can develop a perfect embryo-sac. Until completion of the embryo-sac the ovary and ovule present the same phase of development in both the pollinated and unpollinated flowers, but in subsequent stages there is no noticeable progress of development in the unpollinated flower. Most ovules disappear and only a few survive to develop into embryoless seeds, accompanied by a slight growth of the ovary. The period, at which the fate of ovules in this respect becomes apparent, is after 8-9 days of bloom. Although the full-grown ovary is much smaller than the normal one, yet it is provided with the fruit character (see Text-fig. 15).

In all the stocks used in the present experiment the unpollinated flowers do not drop off and invariably develop fruit and seed. However, certain individual variations may be observed in the number of ovules partaking in the seed formation and in the size of the fruit. On some stocks all the unpollinated flowers produce more numerous seeds than those on other stocks, in spite of similar conditions under which the flowers are developing. Further, it may be probable that the number of seeds correlates with the nutritive condition, under which the unpollinated flower is developing, and which may probably vary according to the number of the accompanying normal fruit produced by pollination. The experiment made in 1910 furnished an example of such a case. When most flowers were pollinated, the ovaries of a few unpollinated ones in the same inflorescence suffered great hindrance in growth and were almost incapable of developing the embryoless seed.

The capsule arising from the unpollinated flower dehisces later than the normal one.

2. THE ABCISCED FLOWER OR FLOWER-BUD.

Having learned in the preceding experiment the normal development of the ovule in intact unpollinated flowers, I now proceed to describe how the ovule of the flower removed from the inflorescence axis behaves in its development.

Experiment 2.

Closed or opened flowers were cut off through their stalk from the inflorescence axis. When kept from drying in PETRI's dish, they could remain alive for a week or more, enabling us to make daily observations on their subsequent development.

1. Flower-buds abscised two days before bloom, while the ovule was at the archesporium stage:—After 4 days the perigone remained closed but appeared fresh. The embryo-sac attained to the two-nucleate stage, with the plasm assembled at the upper portion.

2. Flower-buds abscised a day before bloom, while the ovule was at the archesporium stage:—After a few days they were quite fresh and healthy. The perigone opened, but not so widely as on the intact flower. The ovary grew a little. Having already passed through the megaspore stage, nearly all ovules developed 2- or 4-nucleate embryo-sacs, generally having the plasm massed at the upper portion. When observed after 15 days, the flowers were yet fresh; however, the embryo-sacs had all degenerated. Judging from the disappearance of starch-grains from almost all cells of the ovary and ovules, the flowers were already at this period in a starvation condition.

From these observations it is clear that the ovular development goes on unarrested in abscised flower-buds. Compared with the intact buds, however, its progress appears somewhat slower. An earlier consumption of the nutritive materials would not perhaps enable the embryo-sac to survive for a long time and would render the development of any ovule into seed very difficult.

3. The ovary of opened flowers dissected longitudinally and kept with the placenta, being exposed in a moist chamber:—In this case it was impossible to keep the ovary fresh and alive for a long time. However, so long as it remained fresh, the ovule proceeded normally in its development and passed after 2 days from the archesporium stage to that of the two-nucleate embryo-sac, or from the megaspore stage to that of the four-nucleate embryo-sac, the stage previous to the formation of the egg-apparatus. When the ovary was dissected while the sac was at the two-nucleate stage, the completion of the embryo-sac took place after 2 days.

During the formation of the embryo-sac an excentric assemblage of the plasm often took place in the sac at its two-nucleate stage, sometimes hindering the activity of the lower nucleus. This may perhaps be assigned to an unfavourable nutritive condition, as already remarked.

4. Flowers abscised the day of bloom:—After 3 days the ovary became a little larger than on the preceding day.

After 4 days the ovules completed the egg-apparatus.

After 6 days the sac in most ovules was obliterated and shrank, being

filled with a pale yellowish homogeneous mass. Starch diminished considerably from the ovular tissue.

After 9 days the ovary increased 1.5 or 2 times in diameter as at the stage of bloom, but its cavity appeared disproportionately more hollow and spacious. Most ovules contained shrunken sacs and did not grow further. Very rarely, gigantic ovules were found, which were characterized by having swollen cells.

After 13 days the surviving ovules were exceedingly rare, amounting only from 20 to 30 in each ovary. They acquired a seed-form. Starch was yet found in the ovules and placenta.

After 15 days the surviving ovules developed into adult seeds with rounded ends. The wall of the testa-cells was about to thicken. A few starch-grains were found in the testa-cells. As the ovaries were attacked by mould fungi and begun to decay at this period, a further stage could not be observed.

Results:—The ovary of the abscised flower or flower-bud may attain the size which is reached by the normally developing ovary at its fertilization stage. The ovules are also able to develop subsequently and to complete the embryo-sac. When the flower is abscised on the day of bloom, some ovules, though exceedingly few, continue their development to embryoless seeds.

3. THE FLOWER ON THE UNPOLLINATED STOCK.

The inflorescence of large tubers bears usually more than 50-60 flowers. From an ecological point of view it should be certain, that the quantity of the reserve material stored up in the tuber corresponds with the number of fruit to be produced. Now, removing a large number of flowers from the inflorescence, there would occur a surplus of food for the few remaining ones. What influence this condition of nutrition has upon the flowers allowed to develop further unpollinated, will be seen in the following experiment.

Experiment 3.

Two vigorous stocks were selected for this experiment. The upper portion of the inflorescence axis was cut away, leaving behind only 10-12 flowers, all at nearly the same age. The latter were then left unpollinated, and one or two of them were taken at required periods for the examination of their development phases.

Stock 1 (Text-fig. 17).

After 4 days the perigone was fresh, and the development of the ovary and ovule was similar to that in Experiment 1.

After 5 days the perigone wilted and in the majority of ovules the egg-apparatus was completed ready for fertilization.

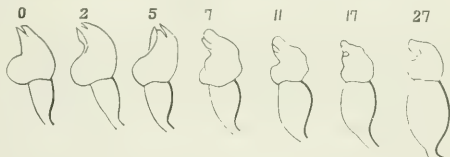
After 7 days the ovary somewhat thickened, but showed no correspond-

ing growth in length as could be seen in the fertilized ovary at the same age. The general structure of the ovule corresponded to that at the 5-days stage of the fertilized ovary, but the exposed portion of the nucellus appeared more projected, and, as a whole, the ovule increased in size, especially in length, due to the corresponding enlargement of the component cells. In some ovules the integument grew up to the level of the apex of the nucellus.

The egg-apparatus was almost all healthy and often the egg-cell was hypertrophied, with an increase of the dense plasma, sometimes so much that it compressed the pole nucleus against the basal wall of the sac.

After 8 days no notable change was visible.

After 12 days the ovary grew very slightly, but not in length. The ovules mostly remained intact, becoming slightly larger than before, while a few of them were obliterated. Among the intact ovules, some showed a gigantic growth accompanied by the growth of the integument, and attained in length twice the breadth. The component cells made also a corresponding enlargement. Many embryo-sacs shrunk and were filled up with a homogeneous, deeply staining mass of disorganized protoplast. The ovules with the obliterated embryo-sac presented a great variation in size. There were also found small ovules possessing the egg-apparatus in healthy condition. The structure of the embryo-sac in the gigantic ovule was quite normal.



Text-fig. 17. A flower from an unpollinated stock. Nat. size.

After 16 days the ovary appeared more swollen. Gigantic ovules became spindle-shaped and somewhat pulverous. The turgescient epidermal cells contained large grains of starch. The surviving ovules were much fewer in number than the obliterated ones.

After 27 days the ovary remained juicy. The gigantic ovule developed into the embryoless seed with normal testa.

After 32 days the capsule begun to delisces. At this period blackened dead spots appeared on the inflorescence axis.

Stock 2.

After 5 days the perigone remained fresh and open, and the ovule exhibited an ordinary course of development, already completing the egg-apparatus.

After 7 days the general feature as in Stock 1.

After 11 days the general feature corresponded to that observed after 12 days in Stock 1.

After 14 days the ovarian development was similar to that of Stock 1. Gigantic ovules of various sizes were spindle-formed or oblong. The swollen epidermal cells contained starch-grains, and their walls did not yet thicken.

After 24 days the ovary thickened considerably, giving a rounded outline

in cross-section. The embryoless seeds were dried up, being easily dispersed on dissecting the ovary.

After 27 days the ovary showed no subsequent growth and remained juicy.

After 32 days the ovary developed into a capsule, still juicy and not attained to the dehiscing stage. The inflorescence axis was quite fresh and healthy. As the axis was cut off on this day, the period of dehiscence of the capsule was not ascertained; probably it would have arrived after 33 or 34 days, if the axis had remained intact.

In the next year the experiment was repeated, yielding similar results.

Results:—The ovary on the unpollinated stock shows a more marked growth than that of the unpollinated flower on the pollinated stock. Its growth endures for a longer period, and the fruit retains the fresh and juicy state for a longer period than that produced, under ordinary conditions, by fertilization. While the ovary of the pollinated flower increases more in length than in breadth soon after fertilization, in the present case the increase in both directions proceeds in the same proportion, so that the resulting fruit appears shorter. It assumes, differing from the normally produced one, a round shape in transverse section, and it possesses a thick wall and placenta.

During the maturation of the fruit the utilized amount of reserve material in the tuber is, as may be expected, very little. It is shown by a narrower hollow space appearing in the tuber, which should be very wide in the ordinary case, and also by the compact peripheral tissue still packed with a large amount of residual starch-grains. Therefore, there may occur in the unpollinated flowers a surplus supply of nutritive materials for the fruit formation.

The number of embryoless seeds contained in a fruit on the unpollinated stock is not invariably larger than in the corresponding fruit on the pollinated stock. Here the ovarian growth does not regularly correspond with the number of seed-forming ovules.

4. THE FLOWER ON THE ABSCISED INFLORESCENCE AXIS.

In the preceding experiment it has been shown that the postfloral development in the unpollinated flowers is closely correlated to the nutritive condition. It suggests further experiments on this point. I take now a portion of the inflorescence axis in which the nutritive connection with the tuber has been interrupted. Putting the cut end in water, it can remain fresh and

healthy for a comparatively long period, enabling the flowers to develop the fruits.

Experiment 4.

1. On inflorescence axes 6-10 cm long, bearing 10-15 flowers opened a day or two after insertion in water, all flowers were left unpollinated.

At an early period the developmental changes of the flower went on similarly to that on the intact inflorescence axis. After 12-13 days the ovary showed an appearance as at the 3 days stage of the normal pollinated flower. While most ovules shrank, a few surviving ones attained a considerable size and assumed an oval form. The component cells showed a remarkable enlargement, especially in a longitudinal direction, yet containing only a small amount of starch.

2. On a similar piece of the inflorescence axis some flowers were pollinated. After 12 days, while the ovary of the pollinated flower grew as large as that at the 5-6 days stage in the normal case of pollination, it remained in the unpollinated one so small as to correspond in size at most to the 2-3 days stage of the ovary developing on the intact inflorescence. The flowers dropped off easily. The ovarian development seemed to be somewhat less vigorous than in the preceding case, being perhaps interfered with by the vigorously growing ovaries of the pollinated flowers.

3. An entire small inflorescence, consisting of about 30 flowers or flower-buds, was placed in the thermostat at 30°C. for 3 days, whereupon the ovaries of older flowers became very slender. The inflorescence was then brought into a laboratory room. After 8 days from the beginning the ovules were yet small and their development was deferred considerably, presenting the megaspore stage, and starch entirely disappeared from their tissue. After 16 days the ovary, remaining small and thin-walled, contained very few gigantic ovules in its comparatively spacious cavity. On this day the ovarian wall began to dehisce. The starvation of the ovary seemed to have been enhanced by keeping the inflorescence for some time in a high temperature.

The above experiment shows that the nutritive condition of the unpollinated flower is rendered remarkably unfavourable, and starvation follows at an early period. In the second case of the experiment lack of nutrition interfered with the growth of the fertilized ovaries, and in the third case a rapid consumption of the food materials for respiration under a high temperature and for nourishing a greater number of flowers. Under such conditions, however, a few ovules can survive for producing seed, at the expense of food materials which would otherwise have been utilized by other ovules and in part by the ovarian wall and placenta. At any rate, the ovary can be said to develop into a capsule of normal structure, notwithstanding its smaller size and slenderness.

Summarizing the results of several experiments on the unpollinated flowers, we may come to the following conclusions :

1. The completion of the embryo-sac takes place in the unpollinated flower. Under favourable conditions the development of the embryo-sac proceeds quite similarly to that of the pollinated flower. Under unfavourable con-

ditions of nutrition there is a tendency of assemblage of the plasm, at the two-nucleate stage, on its upper portion, rendering the complete organization of the sac highly difficult.

2. Generally the egg-apparatus may remain in a healthy condition after 8-9 days of bloom; hereafter it is obliterated in some sacs, resulting in the shrinkage of the ovule, while in other sacs it survives, enabling the ovule to grow further and to develop into the embryoless seed.

3. Even under the most unfavourable nutritive condition (in an abseised flower) the ovary grows to the same size as in the fertilization stage.

4. At about the time the surviving ovules are differentiated the slightly grown ovary begins to acquire an erect position. Such ovary develops into a capsule, smaller in size, but of normal structure.

5. The number of embryoless seeds developed in a single fruit appears in a certain degree proportional to the nutritive condition.

6. The dehiscing mechanism of the capsule is the same as the normal one, but the maturation or dehiscing period is considerably deferred.

7. Such an ovarian development expresses the vegetative (WINKLER) or autonomic (FITTING) parthenocarpy.

VI. Pollinated Flowers under Different Conditions.

In fertilized flowers, under normal conditions, the structural changes ensue upon the ovarian wall, ovular tissue, and embryo-sac parallel to each other in such a way that they come to the end all at the same period. How the development of one portion relates to that of the other, or what relation exists between the act of fertilization and the development of these portions, would be a problem to be answered by experimental studies, in which an analysis of the developmental processes appears to be of great importance. The subjection of the pollinated flowers to different conditions has been attempted for this purpose.

Before describing the experiments I shall first consider the reaction of the stigma to pollination and to the germination of the pollen-grains.

The stigma of *Gastrodia* is situated at the basal portion of the gynostemium and sits directly on the ovary. At the period of bloom its

surface shows a mucilaginous character. It is due, as in other orchids, to the development of the mucous papillae having very conspicuous nuclei. A short conducting canal, which the pollen-tube passes through, is also lined with a similar tissue. As soon as the pollen sends out the tube (a day after pollination), the surface of the stigma swells up, indicating perhaps the reaction of its tissue to the action of the tube. Two days after pollination, we find the tubes penetrating massively into the stigmatic tissue and passing through the conducting canal as a silky bundle. Entering the ovary they run downwards along the ovarian wall between the placentas, but at this period the tubes do not yet reach the basal portion of the ovary, nor do any of them attach their apex to the micropylar end of the ovule. After 3 days some ovules receive the tube, but fertilization is not yet effected. Some ovules become fertilized on the next day. During the elongation of the pollen-tube cellulose plugs are formed in succession towards the younger portion.

1. ACTIVITY OF THE OLD POLLINIUM.

In nature the pollinium may be carried away by insects upon the stigma on different days after bloom. To ascertain whether the pollinium at different ages presents a different behaviour or not as regards fertilization, the stigma of the just-opened flower was pollinated with the pollinium left upon the gynostemium for certain days after bloom. The pollinium, when too old, became more or less pulverous, but for a few days it remained intact, retaining its sticky character.

Experiment 5.

1. The 2-days old pollinium:—Fertilization and the fruit formation took place normally.

2. The 4-days old pollinium:—After 3 days of pollination the ovaries showed a swelling, some remarkably and some slightly. After 7 days some ovaries indicated the normal development for the fruit, while some showed only a slight growth. In the latter case the fertilized ovules were less in number than in the former.

3. The 6-days old pollinium:—After 4 days the ovary somewhat increased in size, but less than in the normal case. After 9 days a little further growth was recognized in the ovary, but in most ovules the embryo formation was unsuccessful; only occasionally multicellular embryos were found.

Results :—The germinating power of the pollen is lost when several days old. A check experiment in this connection shows that the 5-days old pollens failed to germinate in a moist chamber, while the 2-days old ones germinated profusely on the next day. The inhibition of the ovarian growth in flowers pollinated with an old pollinium must be, therefore, ascribed to the failure of fertilization.

We recognize a close relation between the durability of the germinating power of the pollen and the period of withering of the perigone. As we have remarked previously, the perigone remains widely open and fresh until 4 days after bloom, allowing the visit of insects. It would be ecologically important that the pollinium retains its germinating power during this period for rendering pollination effective.

2. PERIOD OF POLLINATION.

It is evident that the development of several parts of the flower is so adapted as to receive the pollinium at the time of bloom. In case of untimely pollination, therefore, the question arises as to its effect towards fertilization, and as to the developmental features of several parts before or after fertilization. HARTLEY'S ('02) experiment in this respect with tobacco plants shows that application of good pollen to immature pistils causes the flower to fall off. In carrying out a similar experiment with *Gastrodia*, the pollinia taken from just opened flowers were applied to the stigma of flowers of different ages.

Pollination of Young Flowers.

Experiment 6.

Pollination was made a day or two before bloom. At the latter period most ovules, as already mentioned, were yet rudimental and presented the synapsis knot stage of the archesporium nucleus, and at the former period the spireme stage. The pollen germinated immediately. Two days after pollination, that is, a day or two after bloom, the embryo-sac was yet at the binucleate stage with its plasm massed at the upper end, while the unpollinated flower in the same inflorescence, becoming 3 days old, had the more advanced sac, being mostly at the 4-nucleate stage, or completing the egg-apparatus. The pollen-tube did not reach the ovule. Thus the development of the pollen-tube and the embryo-sac were essentially normal.

When observed 4 days after pollination, the embryo-sac in the flower pollinated a day before bloom was mostly found to complete the egg-apparatus, and indeed many ovules were already fertilized, while the flower

pollinated 2 days before bloom showed a little earlier stage of the ovular development.

The subsequent development of the ovary and ovule proceeded approximately similar, as observed in flowers pollinated at due time.

The above experiment appears to show that an earlier pollination brings about a little earlier fertilization. Probably it may be ascribed to the developmental condition of the pollen-tube rather than to that of the embryo-sac. For, as already mentioned, in the flower pollinated on the day of bloom the critical period of fertilization is reached after 4 days, in spite of the completion of the egg-apparatus after 3 days. The failure of fertilization after 3 days under ordinary conditions is due to an incomplete development of the pollen-tube. In the present experiment the pollen-tube at the 4-days stage and the ovule at the 3-days stage are brought into combination, both being at the critical period for effecting fertilization.

Pollination of Old Flowers.

Experiment 7.

1 (1911).

Pollinated 2 days after bloom:—The ovary, 3 days after pollination, that is, 5 days after bloom, became slightly larger than that at the 4-days stage in the flower pollinated the day of bloom, and fertilization was taking place in some ovules.

Pollinated 3 days after bloom:—The ovary, 4 days after pollination, showed the normal course of growth and the effect of fertilization. At the 5-days stage (after pollination) it was similar in size to the ovary pollinated on the day of bloom which attained to the 5-days stage, and the embryo showed also a similar stage of development. After 15 days seeds with the typical testa matured. The fact that most ovules were fertilized could be shown by the amount of seed produced.

Pollinated 4 days after bloom:—At this period the perigone remained yet open, but the inner labellum shrank and hid the top of the gynostemium from view. After 2 days the ovary became larger, especially more elongated than in the unpollinated flower. After 3 days the growth of the ovary became more conspicuous. After 7 days the ovary was similar in size to that at the fertilization stage in the normally pollinated flower. Here the number of fertilized ovules was diminished, and as the result the ovary was much stunted in growth.

Pollinated 5 days after bloom:—The perigone already wilted at this period. After 2 days the growth of the ovary was inconspicuous. When observed after 5 days it showed scarcely any subsequent growth. After 6 days most ovules attained the size at the fertilization stage, but in fact remained unfertilized. Seldom two-celled proembryos were found in some ovaries, while fertilization failed entirely in other ovaries, which were as much inhibited in growth as those of unpollinated flowers. After 10 days

most ovules in the unfertilized ovaries shrank, while a few surviving ones showed a gigantic growth.

(2 1912).

Pollinated 3 days after bloom:—The result was essentially similar to that obtained in the preceding year. After 6 days two-celled proembryos were found in some ovaries.

Pollinated 4 days after bloom:—After 4 days the pollen-tube reached the micropylar end of the ovules, but in some ovaries all the ovules advanced in development so far as to be incapable of fertilization. In other ovaries, however, two- or three-celled proembryos were found after 5 days in large number of ovules that assumed a spindle shape. After 11 days few-celled embryos were derived from these proembryos. The ovary containing numerous embryos developed into a normal fruit.

3 (1913).

Pollinated 3-4 days after bloom:—On numerous flowers examined, the ovaries advanced in development, so as to produce essentially normal fruits, pointing out that fertilization took place undisturbed.

Pollinated 8 days after bloom:—Of four adjoining flowers in the same inflorescence two were pollinated and the other two were left unpollinated. At the beginning of the experiment they all showed the same development of the ovary. After 4 days the difference in size between the pollinated and unpollinated ovaries became somewhat apparent (Text-fig. 16). After 6 days some embryoless seeds, already provided with the testa, attained to maximal size. They occurred in abundance. The pollen-tubes penetrated into the stigmatal tissue, but I was unable to follow them in the ovarian cavity. After 20 days the pollinated ovary much exceeded in growth the unpollinated one, but, as a remarkable fact, embryogenic seeds were scarcely found.

Pollinated 10-15 days after bloom:—At this period the surviving and disorganizing ovules had already been differentiated, and the effect of pollination was not presented at all.

4 (1914).

Pollinated 6 days after bloom:—Perigone wilted, but the stigma was yet mucous. After 3 days the ovary did not differ in size from that of the unpollinated flower, but it happened in some stocks that the pollinated ovary took an erect position. After 5 days all the pollinated ovaries increased in size and could be easily distinguished from the unpollinated ones. After 8-9 days they exhibited a certain variation in size; in some it corresponded to the 7-days stage of the normally pollinated ovary, while in others it was much smaller. The upper portion of the ovary was much swelled and towards the basal portion it tapered gradually. Corresponding to this, more numerous seeds were produced in the upper portion, while in the lower their amount was as few as in the unpollinated ovary. Many seeds contained the embryo, but there were, especially in the lower portion, sterile ones. After 13 days the seed matured.

We learn that some ovules retain the fertilizing activity. That the fertilized ovules are more numerous in the upper portion of the ovary may be explained as being due to the arrival of the pollen-tube earlier than in

the lower portion, where the fertilizing activity of the most ovules are lost at the time of arrival of the tube.

Pollinated 7 days after bloom:—At this period the ovules with the shrunken embryo-sac were very few; the egg was hypertrophied and contained a large vacuole; enlarged ovules were rarely found. The difference of the ovary in size from the unpollinated one became apparent after 5 days. After 7 days the growth of the ovary was conspicuous; some ovules, especially at the upper portion of the placenta, were fertilized (40%), and the seed-developing ovules decreased in number in the lower portion. After 9 days the ovary was far smaller than the same-aged one normally pollinated. The amount of seed is a little larger than in the unpollinated ovary (1:1.5); in the pollinated ovary smaller seeds were found, but none in the unpollinated one. The seeds which contained the embryo were few in number.

After 11 days we obtained, besides the enlarged ovaries, some smaller ones, similar in size to the unpollinated ones. The number of seeds in the smaller ovary was approximately equal to that in the unpollinated one. Only a few of them contained the embryo.

After 18 days the capsules derived from the pollinated and unpollinated flowers dehisced at the same time, due to the unhealthy state of the axis. Both capsules were only slightly different in size.

Taken altogether, the results of experiments relating to the period of pollination may be summed up as follows:

When pollination is delayed for 2–3 days, fertilization may take place almost undisturbed, and the formation of fruit proceeds quite normally. In flowers pollinated after 4 days, fertilization is sometimes quite ineffective, and, if it is effective, the resulting fruit varies in size according to the number of embryogenic seeds. A further delay makes the fertilization more difficult, though a few ovules may retain their fertilizing activity as late as 8 days after bloom. Pollination so much delayed that all ovules become quite incapable of fertilization, may induce no promoting action upon the growth of the ovary.

Generally the ovule, like the pollinium, retains the fertilizing activity as long as the perigone is fresh.

A striking feature, found in the series of experiments, is the occurrence of polyembryony. This will be discussed later on.

3. POLLINATION INTO THE OVARY.

STRASBURGER ('86) has already ascertained in some orchids that the insertion of the pollinium into the ovarial cavity may effect fertilization and produce ripe seeds. It shows that the germination of the pollen-tube and the resulting development of the ovary and ovules are manifested without the

agency of the stigma. This is a point worthy of consideration in *Gastrodia*, regarding the relation between pollination and the ovarian as well as ovular development.

Experiment 8.

1 (1911).

The ovary was cut transversally through the upper portion, and the pollinium was introduced into the cavity from the cut surface. After 4 days the ovary showed a normal growth; after 5 days it elongated and swelled up, indicating the accomplishment of fertilization; after 7 days a further growth of the ovary was observed, some ovules were still young and small, being at the fertilization stage, but others became enlarged and somewhat pulverous, developing multicellular embryos. Normal seeds were developed in abundance from the ovules of the latter.

2 (1911).

The pollinium mixed with the mucilage taken from the stigma was introduced into the ovarian cavity from the cut surface. After 3 days the ovary showed a normal growth; after 4 days the pollen-tubes did not penetrate into any of the ovules; after 5 days they elongated further, but no entry into the embryo-sac was ascertained; after 7 days the ovary showed no subsequent growth, yet a small number of ovules were found already fertilized, producing the two-celled proembryo.

3 (1913).

The pollinium was introduced into the ovarian cavity through an opening made on the upper portion of the ovary.

After 3 days the ovary showed no difference in size from that of the same-aged unpollinated flower. After 4 days it showed a slight swelling, and after 6 days it grew remarkably. After 7 days the difference in size became apparent among several ovaries treated similarly. After 10 days the fertilized ovules had grown as large as an adult seed, but without completing the testa. Fertilization was not accomplished uniformly throughout the ovarian cavity. Often most ovules on one placenta remained unfertilized, with a less growth of the ovarian wall on this side. These ovules showed the same phase as those of the unpollinated flower, some being small and having the shrunken embryo-sac, and some becoming gigantic.

Under favourable conditions nearly all ovules were fertilized, and the fruit produced grew as large as the normal one.

After 14 days seed matured, but the fruit-wall was yet juicy.

Results:—The pollinium may germinate within the ovarian cavity and effect fertilization, thus without agency of the stigma. Sometimes the distribution of the pollen-tubes is not universal throughout the ovarian cavity, leading to the failure of fertilization in many ovules. In connection with the limited number of fertilized ovules the resulting fruit is smaller than usual.

4. SELF- AND CROSS-POLLINATION.

Among entomophilous flowers self-pollination may sometimes be ineffective and even the pollen-grains may act injurious on the stigma of their own flower, while in other cases both self- and cross-pollination has the same effect. Besides these extreme cases, both processes of pollination differ in several degrees, regarding fertilization and the development of the fertilized ovary. For instance, FABER ('12) finds in *Cajia*, that in the case of cross-pollination fertilization takes place after 4 days, while it takes place after 6 days in self-pollination. According to v. TSCHERMACK ('02), the growth of the fruit of *Cheiranthus* produced by crossing is more vigorous than by self-pollination.

In *Gastrodia* the effect of self- and cross-pollination was carefully compared with flowers in the same inflorescence. It revealed no difference at all between the two cases; fertilization took place in the same interval (after 4 days), the development of the embryo and fruit was also quite similar in all details. So that, in an entomophilous flower so much adapted in structure for cross-pollination as the Orchidaceae, self-fertilization can be effective quite as well as cross-fertilization. Of course, the flower is constructed so as to ensure cross-fertilization by insect-aid, but occasionally the pollinium may happen, in natural condition, to fall (shaken by wind) upon the stigma of its own flower, which is situated at the base of the gynostemium, and cause self-pollination. In DARWIN's work ('86, p. 290) several orchids are mentioned as being capable of fertilizing themselves without the aid of insects. In some orchids RIDLEY ('88) has noticed the possibility of self-fertilization, though not evidenced by experiment. Perhaps the same thing as in *Gastrodia* might be possible in several other orchids.

5. POLLINATION OF FLOWERS ON THE ABSCISED INFLORESCENCE AXIS.

In an intact inflorescence the flower would withdraw a large amount of food materials from the tuber as soon as it is fertilized. To know how an interruption of the nutritive connection with the tuber interferes with the development of the fertilized flower is the object of the present experiment.

Experiment 9 (Text-fig. 18).

Many inflorescences were used, sometimes an entire inflorescence, sometimes a portion bearing only a few flowers. Therefore, the relation between

the total amount of food materials present in the vegetative tissue and the number of the fruit-producing flowers was variable. Yet, the general course of development was quite similar in all the materials used. On this account, we need mention only the result of observation on an inflorescence in which the successive stages were studied in more details.

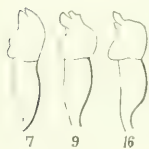
A short piece of an inflorescence axis bearing 7 flowers was kept, being inserted in water, when all the flowers opened at once and were pollinated on the day of bloom.

After 4 days the ovary showed the normal growth and most ovules were in fertilization.

After 7 days the ovary grew further, attaining at the most a size as at the 5-days stage in the normal case. Starch found in the ovular tissue was as abundant as in the normal case. The proembryo gave a row of 3-4 cells and its apical cell underwent the longitudinal division. The growth of the integument proceeded normally and its margin extended a little beyond the nucellus. The ovule assumed a normal, oval form.

After 9 days the ovary remained in size as before. Ovules became seed-formed, but most of them were broader and shorter than the normal ones at the same stage. Starch in the ovule diminished considerably. The embryo became few-celled.

After 12 days no subsequent growth of the ovary was observed. Young seeds were spindle-shaped, but shorter than the normal



Text-fig. 18. A flower on an abscised inflorescence axis, pollinated with the *Gastrodia*-pollinium. Nat. size.

ones at the similar stage, being rounded at both ends. Epidermal cells contained a few large grains of starch and their wall did not yet thicken. The embryo became multicellular.

After 16 days the ovarial cavity was filled up with pulverous, nearly ripe seeds having the testa with the sculptured surface. The embryo was already packed with reserve materials.

As the seeds were produced in normal amount within a far smaller cavity of the ovary, they were arranged more compactly than usual. Compared with the normal seed the tissue of the testa was more or less arrested in development. The lesser elongation of the composing cells at the charazal end and the depressed multiplication of cells in the integument at the opposite end gave a rounded outline to the seed (Text-fig. 19).

In a comparatively large number of seeds the testa consisted of the tissue itself, which had developed up to the time of fertilization (cf. Text-fig. 11). Strictly speaking, no integumental tissue has ever developed in this case, and in becoming the testa the epidermal cells of the ovule merely elongated and enlarged slightly till the reticulate thickening of their wall took place. On this account, at the micropylar end the testa reached scarcely a little beyond the top of the nucellus (Text-fig. 20). On the whole, the development of the embryo was essentially



Text-fig. 19. A young seed from a flower on an abscised inflorescence axis, 16 days after bloom. $\times 200$.

normal, but the ovarian wall and the seed-coat were greatly retarded in development.

After 19 days the capsule came to maturity and dispersed the seed on dehiscence.

Results:—Fertilization takes place normally. The number of ovules developing into the seed is nearly as large as in the normal case. The growth of the ovary after fertilization is greatly hindered and its final size corresponds to that acquired by the ovary at its 5-days stage during the normal development. The resulting smaller capsule dehisces at the ordinary period. Most seeds possess poorly developed testa, owing to a lesser elongation and enlargement of the component cells and also to the perversion of the development of the integument, in spite of the almost normal development of the embryo.



Text-fig. 20. A young seed from a flower on an abscised inflorescence axis, 16 days after bloom. $\times 200$.

As a whole, the scarcity of food material causes an imperfect development of the ovarian and ovular tissue, but it does not interfere with the number of ovules going into seed formation, nor with the development of the embryo.

6. POLLINATION OF ABSCISED FLOWERS.

The flower taken off from an inflorescence axis is in a more unfavourable condition of nutrition than that on an abscised inflorescence axis. While in the latter the food materials stored in the axial tissue may be in part utilized by the fertilized and growing ovary, in the former the supply of the food materials from any extrafloral portion is absolutely interrupted. Under such limited nutrition the flower can remain alive till the fertilized ovule develops into the seed.

Experiment 10.

Just opened flowers were cut off as soon as pollinated, and were kept in PERRI's dish. Care has been taken not to keep the medium too moist, since, otherwise, the flower might be attacked by mould fungi.

1 (1911).

After 3 days the ovary swelled as on an intact flower; the development of the ovule proceeded normally; pollen-tubes elongated so much as to attach to the micropylar end of the ovule.

After 5 days the ovary was smaller than the normal one at the 4-days

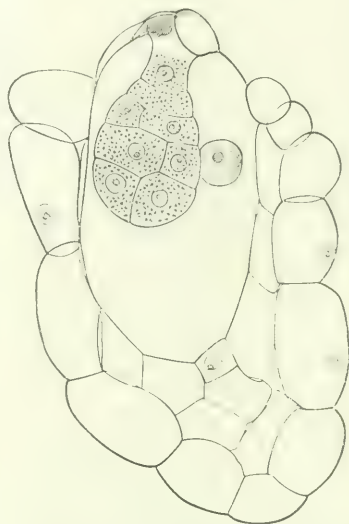
stage; the ovule increased in size as at the stage of fertilization. Many ovules contained two- to four-celled proembryos, but there were yet unfertilized ovules provided with the complete egg-apparatus or with degenerated embryosacs. In fertilized ovules the integument was greatly hindered in development, whereas the ovule remained in an oval form.



Text-fig. 21. The 7-days stage of an ovule from an abscised flower, pollinated on the day of bloom (living specimen in optical section). $\times 400$.



Text-fig. 23. The 20-days stage of the same (living specimen in surface view). $\times 400$.



Text-fig. 22. The 15-days stage of the same (living specimen in optical section). $\times 400$.

After 6-7 days the ovary attained the size corresponding to that attained by the normal pollinated flower after 3-4 days. In microtome sections we learned that, accompanying the hindrance of enlargement of the ovarial cavity, the placenta showed no such growth as could be seen in the normal case. Under this condition the fertilized and growing ovules were arranged compactly. The development of the integument was much prevented and also the embryo-sac remained small, though the proembryo grew undisturbed, so much as to fill up nearly the cavity of the sac.

2 (1913).

After 4 days the ovary became larger than on the abscised day, but it was smaller than the intact one at the same age; the perigone remained fresh; most ovules were in fertilization.

After 7 days starch disappeared almost entirely from the ovule, giving a transparent view to it; the proembryo attained to the two-celled stage (Text-fig. 21), occupying the greater portion of the small cavity of the embryo-sac. While in the normal, pollinated flower the integument developed considerably at the corresponding stage, here its marginal growth was suppressed entirely, so that the nucellus remained exposed just as at the fertilization stage. The component cells of the ovule underwent no enlargement (compare Text-figs. 13 and 21).

After 9 days a remarkable differentiation took place among the fertilized ovules. Many ovules remained as at the 7-days stage, containing mostly collapsed embryo-sacs, but some became strikingly hypertrophied with their component cells swollen and turgescient, and also with the enlarged embryo-sac, which contained the 4- to 6-celled and club-shaped proembryo. The hypertrophied ovules were as large as observed in the unpollinated flowers in the preceding experiment.

As a most noteworthy fact we observed that, while starch disappeared entirely in the collapsing ovules, a certain amount of it remained in the component cells of the hypertrophied ovule. It gave an indication that there prevailed a struggle for existence among the fertilized ovules at a certain stage after fertilization, and that the surviving ovules withdrew the nutritive material from the perishing ones.

After 11 days no noteworthy change occurred subsequently in the ovule, except that the proembryo grew further. Large starch-grains were still found in the integumental cells.

After 15 days many ovules shrank entirely, but the surviving ones proceeded for the seed formation, producing multicellular embryos. Ovular cells, especially the epidermal cells, were strongly turgescient, containing sometimes a residue of starch, though in most ovules they became quite empty of it (Text-fig. 22).

Generally, the development of the ovular tissue was checked, no cell multiplication taking place from the seventh day on. At this stage the nucellus remained mostly exposed. The ovule was generally far broader and shorter, taking on an oval form. No thickening of the wall took place in the testa-cells.

After 17 days the seed assumed a broad spindle form with rounded ends; the embryo acquired the adult state, being densely granular due to accumulation of reserve materials. The reticulate thickening already took place or was taking place on the wall of the testa-cells.

After 20 days the ovary remained in size as at the 4-days stage of the normal one, and was yet fresh. However, its cavity was filled up with greyish dried seeds which corresponded in general appearance to those found in a mature fruit developed under the normal condition. The seeds were pulverous, with the testa and embryo completed in their development. The maturity of the embryo was indicated by a yellowish colour and compact structure, though it was generally smaller (Text-fig. 23).

Most seeds showed more or less deviation in form and size; they were broader and shorter. Among them we found some, whose seed-coat was so greatly suppressed in development that we might almost say, the embryo was without the seed-coat (compare Text-figs. 23 and 27).

The ovarian wall assumed the structure of the capsular wall but was incapable of dehiscence.

3 (1913).

Another set of flowers treated similarly yielded the same result. Observed after 16 days the seeds in the upper half of the ovarian cavity became greyish, showing a complete maturation, while those in the lower half were at a little younger stage, with complete testa and a full-grown yellowish embryo. At this stage a residue of starch was found in the placental tissue, so that the fruit was not in fact at an entirely starved condition at this period.

Results:—Fertilization is not prevented. After fertilization the growth of the ovary does not take place. All fertilized ovules can develop up to a certain stage (generally the stage developing the 2- or 3-celled proembryo); hereafter some ovules disintegrate and only the surviving ones succeed in developing the seed.

In most ovules the integumental tissue does not, in a strict sense, come to development. It results in the formation of the seed with such an imperfect seed-coat that the seed often appears to be represented by the embryo itself.

Scarcity of the nutritive materials, to which the abscised flower is subjected, causes no or imperfect development of the ovarian and ovular tissue, while the embryo is not essentially affected in its nutrition and development.

7. POLLINATION OF FLOWERS WITH BROKEN OVARIES.

Experiment 11.

1. A transverse hole, 1mm. in diameter, was made on the ovary by piercing it with a thick needle. Pollination induced a normal growth to the ovary and effected fertilization to the ovules, except those along the hole, resulting in the formation of the capsule with ripe seeds. If the hole was large, the capsule was much deformed and small.

2. When the ovarian wall was split along 2 or 3 longitudinal lines, fertilization was not hindered in the exposed ovules. However, the ovary could not grow normally, the resulting capsule being much smaller in size (the 6-7-days stage of the normal ovary). The seed, though normal in size, appeared incapable of full maturation.

3. When one of the three placentas was taken off together with the ovarian wall, the other placentas became exposed. Fertilization was largely disturbed, as the ovules shrank previously, or the fertilized ovules perished during the subsequent development. Still we could obtain a few mature seeds in a small deformed capsule.

4. A transverse notch was made at various heights of the ovary, reaching to its median longitudinal axis, and a piece of a cover glass was inserted in the wound thus produced. Pollination induced the undisturbed growth of

the ovary. But the portion of the ovary below the notch was retarded in growth, and the ovules under the cover glass remained mostly unfertilized. As a whole the capsule could attain to normal size in a deformed form.

5. A red-hot needle was inserted longitudinally into the ovarial cavity. Almost all ovules thus perished. Pollen-tubes entered the ovarial cavity, but they could not exert stimulation to growth. Yet the ovarial wall remained fresh and healthy, though fertilizable ovules were lacking. On the other hand, if a few ovules were left alive, more or less growth took place on the ovarial wall, accompanying the development of the seed.

Results :—The wounded ovary is able to develop into the capsule, allowing the living and fertilized ovules to form seed. As a remarkable fact, the pollinated ovary is unable to grow further, when all the ovules are killed, in spite of the germination of the pollen on the stigma and the entry of the pollen-tube into the ovarial cavity.

VII. Stimulus upon the Stigma.

FIRTING'S ('09, '10) extensive experiments on orchid flowers have brought about several interesting results, among which we find the most interesting fact that the stimulus in various ways upon the stigma results in the inducement of several different phenomena of postfloration. Following him I tried a few experiments with *Gastrodia* respecting the problem of postfloration; in particular, I paid special attention to the relation between the stimulus upon the stigma and the development of the ovary and ovule. In several check experiments I came to negative results and the experiments were not extended further. In the following I shall give only the results of a few.

When particles of soil were put on the stigma of a just opened flower, the perigone wilted after 4 days. The growth of the ovary and the development of the embryo-sac showed no divergence from those in the normal case.

When an extract of the *Gastrodia*-pollinium, obtained with boiling water, was put on the stigma, no effect was observed, the flower proceeded in development quite similar to the unpollinated flower.

When particles of cane sugar were applied, the perigone wilted after 5 days. The development of the ovary went on similarly as in the unpollinated flowers.

When particles of sodium chloride were applied, the effect appeared too violent. The stigmatal tissue shrank and blackened. On the next day some

flowers wilted and hung downwards. After 2 days they dried up. In other flowers the junction between the perigone and the ovary shrank. The healthy portion of the ovary could develop like that of the unpollinated flower.

When the stigma was wounded by the pincette the perigone wilted after 3-4 days. After 5 days the egg-apparatus was completed. Subsequently, the wound was healed and the ovary developed like that of the unpollinated flower.

VIII. Pollination with Foreign Pollen.

The action of foreign pollen-grains, not only from the same genus but also from widely diversified families, upon the development of the ovary or ovule has attracted attention since olden times (GÄRTNER, '49). In orchids, HOMMESTER (GOEBEL, '01, p. 793), HILDEBRAND ('63), STRASBURGER ('86), and lately SHARP ('12) obtained the result that the ovule, which is yet rudimental at the flowering stage and remains undeveloped at the prevention of pollination, undergoes a further development, if certain foreign pollen-grains, of course incapable of fertilization, germinate on the stigma. In certain cases an accompanying growth of the ovary is observed. These facts place a remarkable significance upon pollination as implying double action of the pollen-tube, one relating to the development of the embryo and the other to the development of the ovary and ovule (see a review of v. TSCHERMACK, '02).

As orchids afford the best-suited material for the experimental study on this subject, and as the results of the previous investigations suggest more exhaustive researches, an attempt has been made with *Gastrodia* to study in detail the action of the foreign pollens and their final effect upon the ovarial and ovular development. Among several plants which furnished the pollen, *Bletia hyacinthina* brought about the most remarkable results, so that the experiment with its pollen was carried out more extensively than with others.

1. POLLINIUM OF BLETIA HYACINTHINA.

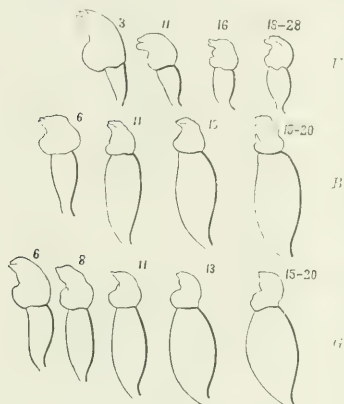
Experiment 12.

Intact flowers.

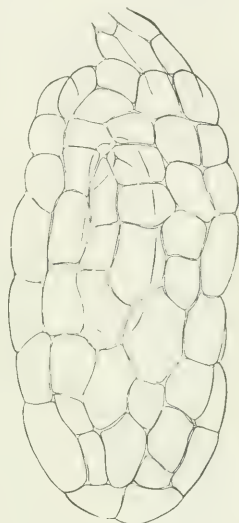
1. To follow out the developmental features of flowers pollinated with *Bletia* in comparison with those unpollinated and pollinated with its own

pollinium, I took first an inflorescence of *Gastrodia elata* forma *viridis*,¹ a bluish green form (Text-fig. 24). For brevity I used the sign *U* for the unpollinated flower, *G* for the flower pollinated with its own pollinium, and *B* for the flower pollinated with the *Bletia*-pollinium.

After 3 days *B* showed like *G* a response to pollination by wilting its perigone, while in *U* it remained fresh. The pollinium of *Bletia*, kept in a moist substratum, germinated massively and vigorously on the third day, sending out long tubes. Germination went on similarly through the stigma, so that on this day already the tubes were found distributed in the ovarian cavity.



Text-fig. 21. Three flowers of *Gastrodia elata* forma *viridis*. *U*, unpollinated; *B*, pollinated with the *Bletia*-pollinium; *G*, pollinated with the *Gastrodia*-pollinium. Nat. size.



Text-fig. 25. A medium-sized ovule in a flower 8 days after pollination with the *Bletia*-pollinium (fresh material). $\times 400$.

Reacting to the germination of the pollen the surface of the stigma showed a swelling just as seen with the pollen of *Gastrodia*. In *B* the ovular development was usual; a few ovules already completed the egg-apparatus, some were at the 4-nucleate stage, some still at the dividing stage of the two nuclei into four.

After 4 days the ovary thickened further both in *B* and *G* similarly, but far less in *U*. The perigone of the last flower wilted on this day.

After 6 days the ovary of *B* and *G* showed an equal increase in size. The ovule in *B*, having a distinct egg-apparatus, acquired a form as was attained in *G* after 4 days. It was remarkably greater than the same aged one in *U*, in which the embryo-sac exhibited the same stage of development.

1. It possesses a smaller capsule than the type.

After 8 days the ovaries of *B* and *G* were of quite the same size, thus showing *B* to be advancing in the fruit formation. In some ovaries of *B* most ovules assumed an oval form with the integument growing up to the level of the nucellus, but in other ovaries some ovules gave indication of a gigantic growth (Text-fig. 25). The largest ovule became twice as large as that found in *G* after 4 days, with a corresponding enlargement of the component cells. The size of the ovule presented at this period a wide range of variation. In the enlarging ovules the embryo-sac remained healthy, the egg-cell being enlarged and the pole nucleus becoming conspicuous with an increase of the chromatic content. The pole nucleus was often found compressed at the base of the sac cavity from the enlarging egg-cell.

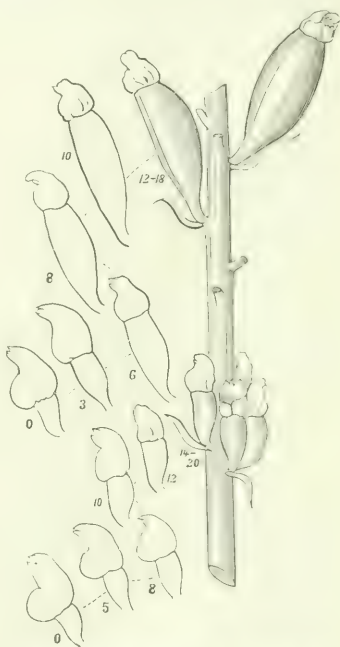
After 12 days the growth of the ovary and of the gigantic ovules in *B* proceeded further.

After 15 days the gigantic ovules presented a wide degree of variation in size. Some already attained the size of an adult seed, but both ends were less tapering. The testa-cells did not yet thicken their wall and still contained a large amount of starch.

At this period the ovaries of *B* and *G* attained their maximal growth into a capsule. An external difference was scarcely recognizable between the capsules; they merely appeared in *G* solid and thicker, provided with a thicker placenta.

After 19 days the surviving ovule in *B* developed into a mature embryoless seed. The size and form were essentially the same as those of the normal seed.

2. The experiment was repeated with *Gastrodia elata*, yielding on the whole the same result. One of the stocks used is reproduced in Text-fig. 26, which bore only 6 flowers. One (left) of the upper two flowers was pollinated with the *Bletia*-pollinium and the other (right) with its own pollinium on the same day, while the lower four were left unpollinated. The difference in growth of the ovaries between the upper and lower sets of flowers became more and more marked on the succeeding days. The mature fruits from the upper two flowers were externally so similar as to be scarcely distinguishable.



Text-fig. 26. Development of the fruit from flowers unpollinated (lower four), pollinated with the *Gastrodia*-pollinium (upper right), and pollinated with the *Bletia*-pollinium (upper left). Nat. size.

The dehiscence of the capsule took place after 28-29 days under a somewhat unhealthy condition of the axis. The seeds produced were as many as in the normal capsule, and in form and structure they revealed in general no difference from the normal embryogenic seeds (Text-fig. 27), though a certain number of smaller seeds were found mingled together.

3. To determine the dehiscing period of the capsules of different kinds, several flowers from a vigorous stock were treated as before. The flower shoot remained quite healthy until all the capsules came to dehiscence. The result was as follows:

	Dehiscing period (days after bloom).
Normal capsule	19
Capsule arising from the unpollinated flower.....	27
Capsule produced by crossing with <i>Bletia</i> -pollinium.....	29-30

The *Bletia* - pollinium is characterized by producing pollen-tubes exceedingly longer than those of *Gastrodia*, forming, like the latter, cellulose plugs. As a most noteworthy fact, they endured exceedingly long and, at the dehiscing period of the capsule, were found still thriving, entangled like fungus-mycelia, throughout the cavity of the capsule. Certain deformations were observed in the tube, giving a node-like swelling and a knee-like bending. In no case the attachment of its apical portion to the micropylar end of the ovule could be observed.

Results :—Towards the fructification of *Gastrodia* the pollinium of *Bletia* behaves quite similarly to that of *Gastrodia*, the only difference being the absence of an embryo in the seed. It is remarkable that the dehiscing period of the capsule is much later.



Text-fig. 27. Full-grown embryogenic and embryoless seed. $\times 150$.

Experiment 13.

Flowers on the abscised inflorescence axis.

A portion of an inflorescence axis, measuring about 10 cm. in length and bearing 7 flowers, all opened a day before, was inserted in water at once, soon after pollination. The ovary increased in size day after day. After 6 days it elongated as much as the same-aged one, which developed on the flower pollinated with the *Gastrodia*-pollinium under the same condition (see Experiment 9) and, though somewhat slender, it corresponded in size to that at the 5-days stage in the normally pollinated flowers. The ovule developed its integument so much that it slightly exceeded the level of the apex of the nucellus. The embryo-sac was obliterated. Starch disappeared from the ovular cells, only a small quantity being left in cells below the funiculus. The intricate pollen-tubes were found among the ovules.

Gigantic development of the ovule was exceedingly rare (only a small percentage).

After 13 days the ovary scarcely corresponded in size to that of the normal fertilized flower at the 6-days stage. The ovarian wall was comparatively thin and juicy, and contained a small amount of starch. Its cavity appeared more spacious than usual. The surviving gigantic ovules developed into nearly mature seeds, some larger, some smaller, as might be seen from the structure of the testa in the progress of development, while others shrank entirely. Pollen-tubes were yet thriving throughout the ovarian cavity.

After 17 days the ovary remained in its general aspect as before. So also the pollen-tubes. The testa was completed.

After 19 days the ovary produced a ripe capsule. The living pollen-tubes, having developed profusely, bound up the pulverous seeds.

As the result of this experiment we know that the growth of the ovary is arrested at an earlier period, and the amount of seed is diminished. As a whole, it appears to show that an imperfect nutrition arrests the promoting action of the *Bletia*-pollinium upon the ovular and ovarian development.

Experiment 14.

Abscised flowers.

After 6 days the ovary scarcely attained in size the fertilization stage. Starch diminished considerably from the ovary. The integument grew nearly up to the level of the apex of the nucellus. Very few ovules became gigantic and oval. Large grains of starch were present only in these ovules. This feature of starch distribution appeared to show that the scanty nutritive materials contained in the flower were supplied chiefly to a less number of ovules to enable them to further develop by sacrificing other ovules.

After 13 days the ovary remained in size as before. Long, intricate, living pollen-tubes were found traversing throughout the ovarian cavity.



Text-fig. 23. A flower on an abscised inflorescence axis, pollinated with *Bletia*-pollinium. Nat. size.

Reticulate thickening was beginning to appear on the wall of the testa-forming cells of the surviving ovules; the cells, however, were in a swollen condition, still containing starch-grains in abundance. The seed attained nearly the maximal size.

After 19 days the ovary matured as a capsule, forming the longitudinal dehiscing fissures in the usual way. The maturation of seed in the cavity of the capsule was shown by its pulverous nature and greyish colour. At this period the pollen-tube was yet alive.

The experiment shows that the ovary is almost incapable of growth, and the amount of seed is reduced more than in the preceding experiment with flowers on an abscised inflorescence axis, but appears a little larger than in the abscised unpollinated flower (see Experiment 2).

Experiment 15.

Pollination into the ovary.

The ovary was cut transversally at its uppermost portion so deep that the wound reached the central axis (compare Experiment 8, case 3), and the pollinium was inserted in the wound.

After 3 days the ovary assumed the same size as in the unpollinated flowers.

After 4 days the ovary became larger than the unpollinated one at the same age.

After 9 days the size of the ovary presented individual variations in wide range; some attained the normal size as might be seen in the case of pollination on the stigma, while some increased only a little in size after the 6-days stage.

After 10 days the pollen-tubes, examined in a medium-sized ovary, ran downwards along the ovarian wall; most of the ovules grew up in oval form, a few of them more elongated and becoming seed-formed.

After 12 days larger and smaller ovaries were examined. In the smaller ovary the development of the pollen-tube was less vigorous than in the larger one, showing the fact that the size of the ovary is proportional to the intensity of the stimulus of the pollen-tube. The larger ovary corresponded in size to the same-aged ovary pollinated on the stigma. In both ovaries the large embryoless seeds were developed massively just below the wound, that is, at the portion of the placenta nearest to the pollinium applied, showing an intense stimulus being excited by the tube at this portion. Towards the lower portion of the placenta the amount of seed decreased remarkably equal in both ovaries, amounting in the smaller ovary to ca. 10 per cent. and in the larger to 30 per cent. of the ovules.

In proportion to the decrease of the amount of seed towards the lower portion of the placenta, the ovary showed a tapering towards its lower portion, thus giving a somewhat different form from the usual one. This was shown more pronouncedly in the smaller ovary, but in the larger one this was not always the case and sometimes its lower portion, in spite of the lesser amount of seed produced, showed the usual swelling.

After 15 days a smaller ovary exhibited the same feature as before.

Large seeds were produced more numerous just below the stigma than at other portions where their amount was smaller than usual, but sometimes a little larger or smaller than in the unpollinated flower. In all the cases we got a larger ovary than in the unpollinated flower. This would give evidence for the fact that the pollen-tube promotes the ovarian development unparallel with the number of the seed-forming ovules.

Results:—The pollinium of *Bletia* inserted in the ovarian cavity either succeeds quite well, or is more or less disturbed, in germination. The growth of the ovary for the fruit is proportional to the vigour of the pollen-tube within the ovarian cavity. The promoted growth of the ovary due to the stimulating action of the pollen-tube does not necessarily accompany the production of a larger number of normal-sized seeds.

Experiment 16¹.

Old flowers.

1. Pollinated 4 days after bloom:—After 3 days the ovary became a little larger than in the unpollinated flower. Hereafter the promoted growth of the ovary became more conspicuous day by day, but after 11 days it was still somewhat smaller than the same-aged one which was pollinated the day of bloom. 50–60 per cent. or more of the ovules developed into larger and smaller seeds, while in the unpollinated ovary in the same inflorescence only 20 per cent. developed into the normal-sized seeds. The number of seeds diminished remarkably towards the lower portion of the placenta. The development of the pollen-tubes was so prominent that it could be recognized with the naked eye. After 13 days a subsequent growth of the ovary scarcely occurred.

2. Pollinated 5 days after bloom:—The promotion of the ovarian development proceeded quite similarly, as in the preceding case. After 10 days we obtained the ovary corresponding in size to that pollinated the day of bloom. The normal-sized seed, produced in approximately the same amount as in the unpollinated ovary or twice as numerous, came nearly to maturity. However, as a distinction between the two ovaries, we found in the pollinated ovary still smaller ovules in abundance, giving an indication of developing into seeds, while in the unpollinated one the smaller ovules were in fact all obliterated. After 12 days the ovary remained in size as before. After 19 days only a little growth of the ovary took place. It was still juicy and contained smaller and larger ripe seeds, larger in number than in unpollinated flowers. The production of seeds was, however, exceedingly scanty as compared with that in the capsule derived from the flower pollinated with the *Bletia*-pollinium on the day of bloom, in spite of the equally vigorous development of the pollen-tube.

3. Pollinated 6 days after bloom:—After 3 days, though slight, the promotion of the ovarian development was visible. After 5 days it became more prominent. After 7 days the size of the ovary was somewhat smaller

1. Compare Experiment 7.

than the usual one, but it became 1.5 times larger in breadth and length than the unpollinated one. The larger seeds amounted in total to about 300 in number, while the unpollinated ovaries in the same inflorescence developed only 50-60 larger seeds. The pollinium applied on the stigma, though its germination was evident, did not send out into the ovarial cavity so massive, mycelia-like tubes as seen in the case of the earlier pollination, showing that the development of the pollen-tube was inhibited in some way. After 11 days the ovaries which did not show any subsequent growth were examined. All the ovules had shrunk entirely, except those developed into seeds which had already attained the adult stage. Their number scarcely exceeded that in the unpollinated ovary. Though few, the pollen-tubes were found apparently traversing the ovarial cavity. Here it was shown that the pollen-tubes have promoted the development of the ovarial wall, in spite of the amount of seed very slightly exceeding that in the unpollinated ovary. After 15 days the capsule approached the dehiscing stage under an unhealthy condition of the inflorescence axis. The capsule thus derived was slightly larger than that derived from the unpollinated flower.

Results :—The stimulating action of the *Blelia*-pollinium on the ovarial and ovular development in old flowers is largely diminished in spite of a vigorous development of massive pollen-tubes. However, the size of the resulting capsule and the number of seed contained exceed those in unpollinated flowers.

Taking the facts gained from the above experiments all together, we may arrive at the conclusion that, except the omission of the embryo formation, the flower pollinated with the *Blelia*-pollinium presents quite the same phase of development as with the *Gastrodia*-pollinium itself. Under favourable conditions the resulting fruit acquires the same structure and size as the normal one. Insufficient nutrition induces first the inhibition of development on the ovary and then interferes with the number of seed-developing ovules : while in the case pollinated with the *Gastrodia*-pollinium itself it is the embryo that is least interfered with in development by the nutritive condition ; the least influence of nutrition is shown in the present case in the seed-coat.

The postfloral development induced by the *Blelia*-pollinium affords an example of the stimulative (WINKLER) or autonomic (FITTING) parthenocarpy, which results in the formation of a larger fruit than in the vegetative or autonomic.

2. INFLUENCE OF THE *BLETIA*-POLLINIUM UPON THE ACT OF FERTILIZATION OF THE *GASTRODIA*-POLLINIUM.

After learning in the foregoing that the pollinium of *Bletia* behaves quite similarly, except the act of fertilization, to that of *Gastrodia*, I call attention to the point how the pollination by *Bletia* acts upon the fertilization induced by the *Gastrodia*-pollinium itself. Following the result of the hybrid-experiments in *Nicotiana* by GÄRTNER, FÖCKE ('81, p. 448) states, "Der zugehörige Pollen vollzieht die Befruchtung schneller als fremder und erweist sich als allein wirksam, wenn er gleichzeitig mit anderen Pollensorten auf die Narbe gelangt. Auch noch nach Verlauf einer gewissen Zeit vermag der zugehörige Blütenstaub jede Wirkung des früher auf die Narbe gebrachten fremden zu verhindern, später aber nicht mehr." STRASBURGER ('86) has already shown in *Orchis*-species that by applying both foreign and its own pollinia simultaneously to the stigma, the fertilization act of its own pollinium is not by any means arrested. On coming together upon one and the same stigma, two different pollens may give a mutual action in several ways. Theoretically it may be presumed that the period of germination and the rate of growth of the tube is concerned with the respective activity of the pollens.

In the foregoing experiment it has already been ascertained that the *Bletia*-pollinium germinates on the stigma of *Gastrodia* and develops the pollen-tubes quite similarly, as the *Gastrodia*-pollinium does. So that, in studying the action of the *Bletia*-pollinium upon the *Gastrodia*-pollinium, it seemed to be of interest to follow out the events when both pollinia are applied at different periods on the same stigma.

Experiment 17.

Both pollinia were applied on the same day.

Pollination was made on the day of bloom.

After 3 days the ovary enlarged normally and the stigma showed a swelling, responding to the germination of the pollen.

After 4 days the ovary presented the general feature as might be seen at the same period when the *Gastrodia*-pollinium alone was applied. The ovule showed the normal process of development: some ovules were fertilized, some not yet.

After 5 days the ovary showed the normal growth.

After 7 days the ovary became still larger; the ovule assumed mostly an oblong form, with a still smaller embryo.

After 9 days the ovary attained the size of a normally fertilized one.

After 10 days the ovarial cavity was packed compactly with immature seeds. The formation of the testa was not yet completed. All seeds contained each an embryo, yet small.

After 14 days embryogenic seeds matured, though not dried up. Capsule, though attaining the maximal size, was yet juicy.

After 17 days the seed dried up, and became greyish and pulverous.

After 20 days the capsule dehisced together with the normal capsule.

On comparing with the fruit developed on the same inflorescence axis by applying its own pollinium alone, a remarkable difference was found in the number of ovules developing into the embryogenic seeds. While in the latter fruit the ovules, which were in greater part fertilized, derived a considerable amount of seeds all full grown, the fruit produced by applying both pollinia contained numerous small, sterile seeds; the normal embryogenic ones amounted only to 40-60 per cent.

Both fruits were quite the same in size, but in one the normal seeds were less in number. This fact evidently shows that the *Bletia*-pollinium inhibits the fertilizing action of the *Gastrodia*-pollinium, but notwithstanding that, the development of the ovarial wall goes on quite normally. Here the lesser production of fertile seeds is supplemented by sterile ones.

Experiment 18.

The Gastrodia-pollinium was applied two days later than the Bletia-pollinium.

The ovarial development proceeded parallel with that in the flower crossed by *Bletia* alone.

Observed 6 days after pollination by *Gastrodia*, the ovule grew to an oval form with the corresponding growth of the integument. Attachment of the pollen-tube to the micropylar end was not observed. Perhaps no luxuriant germination of the *Gastrodia*-pollen took place.

The observation on the succeeding days revealed the fact that fertilization was accomplished in some, but not in other ovaries. Thus, observed after 10 days, the ovules in some ovaries were mostly fertilized and already developed young seeds, which assumed the final form and the maximal size, but with the testa still incomplete and the embryo yet small. The embryogenic seeds were found uniformly distributed throughout the ovarial cavity.

In another ovary, after 12 days, the number of ovules developing into embryogenic seed was found to be very few, amounting only nearly to 10 per cent. Numerous ovules, however, survived, without obliteration at all, for developing into smaller sterile seeds. The ovary, having passed 14 days after pollination by *Bletia*, became maximal-sized, but on account of less fertility of the ovule it was not so thick as the ovary pollinated normally by

Gastrodia, though its length was nearly equal. After 18 days the capsule dehiscid.

In this experiment it becomes apparent that the *Bletia*-pollinium interferes with the activity of the *Gastrodia*-pollinium.

Experiment 19.

The Gastrodia-pollinium was applied 3 days later.

After pollination by *Bletia* the ovarian development went on normally. Hence, at the time the *Gastrodia*-pollinium was applied, the ovary had swollen in the usual degree. The effect of the latter pollinium varied with individuals of the ovary.

In an ovary observed 5 days after pollination by *Gastrodia*, the ovules assumed an oval form all in similar size. No entry of the pollen-tube into the embryo-sac could be ascertained.

In an ovary, after 7 days, the ovular development presented a great variation; some ovules attained nearly the adult form of the seed, but without the completion of the testa, while some remained smaller.

The development of the embryo in any ovule was obscure, only it was ascertained that larger ovules contained undivided eggs, pointing to the fact that no fertilization took place.

In an ovary, after 8 days, most ovules lying just below the stigma developed into the embryogenic seed, but those situated at the lower portion of the ovarian cavity were by the greater part unfertilized, and were developing into various-sized embryoless seeds.

After 10 days (13 days after pollination by *Bletia*), the ovary attained the maximal size, but it was characterized in appearance by possessing a smaller diameter than that developed from the fertilized flower. The maximal-sized ovules acquired the character of adult seeds, but all lacking the embryo. Such seeds amounted to 20-30 per cent. The remaining ovules were not quite obliterated and gave an indication of development into smaller sterile seeds. The number of larger seeds produced in each ovary were not constant throughout all individuals; they amounted in other ovaries to 30-40 per cent. As a striking feature, I could ascertain that in a similar-sized ovary the pollen-tubes of *Bletia* developed more profusely, and then the number of ovules becoming larger, seeds exceeded the former. This seemed to show that the number of seed, developing due to the action of the *Bletia*-pollinium, is proportional to the number of the pollen-tubes and to their activity. In connection with this it was ascertained that, in spite of the variable number of embryoless seeds, the stimulus given by the *Bletia*-pollinium results in the formation of quite similar-sized capsules.

From this experiment it may be concluded that the fertilizing action of the *Gastrodia*-pollinium is greatly disturbed by the preceding germination of the *Bletia*-pollinium, in as early as 3 days. Perhaps the massive tubes of *Bletia*, occupying the conducting canal of the gynostemium, hinder the passage

of the succeeding *Gastrodia*-tubes, or else the condition of the stigma becomes unfavourable for the germination of the *Gastrodia*-pollinium.

3. THE POLLENS OF OTHER PLANTS.

Experiment 20.

1. *Coclogyne Massangeana* (Orchidaceae):—After 2 days the pollinium applied to the stigma of *Gastrodia* made a swelling; after 3 days the ovary appeared to become a little larger than in unpollinated flowers; after 4 days it grew as large as that pollinated with its own pollinium; after 6 days it scarcely showed a further growth; after 8 days the pollen-tubes developed massively within the ovarian cavity and the ovary attained the 5-days stage of that pollinated with its own pollinium; after 10 days a further growth of the ovary was observed; after 16 days the ovary was far smaller than the same-aged one pollinated with the *Gastrodia*- or *Bletia*-pollinium. The number of the embryoless seeds was about twice as large as in the unpollinated flowers. After 17 days the formation of the testa was completed. After 19 days no development of the ovary was visible. Unlike the case with the *Bletia*-pollinium the seeds were all equal-sized. Pollen-tubes were clearly recognized traversing among ovules throughout the cavity of the ovary.

2. *Oncidium flexuosum* (Orchidaceae):—Its pollinium apparently induced promoted growth to the ovary of *Gastrodia*, but in a less degree as compared with the case of the preceding orchid. Only a few pollen-grains germinated, but the entry of the pollen-tubes into the ovarian cavity was scarcely observable. As the plant died during the experiment I was not able to follow out the events further.

3. *Cypripedium caudatum* (Orchidaceae):—The pollinium applied to the stigma remained quite ungerminated, so that no action was recognized; the ovary presented a similar development as observed in unpollinated flowers.

4. *Lycaste Deppei* (Orchidaceae):—The promoting action of its pollinium upon the ovarian development was presented first 6 days after pollination; the ovary attained to the 3-4-days stage of that pollinated with its own pollinium. Hereafter a further growth of the ovary took place, but less conspicuous. The pollinium germinated a little, but the pollen-tubes were not found within the ovarian cavity.

5. *Lilium tigrinum* (Liliaceae):—The pollen-grains caused a swelling to the stigma of *Gastrodia*. After 3 days they sent out short pollen-tubes; after 10 days germinated grains increased in number, but there was no sign of penetration of the tubes into the ovarian cavity. The growth of the ovary went on similarly as in the unpollinated flowers. After 17 days adult embryoless seeds were obtained in the same amount as in the unpollinated flowers.

Thus the germination of the *Lilium*-pollen on the stigma of *Gastrodia* took place with difficulty, and its effect upon the ovarian development was scarcely recognizable.

6. The pollens of other plants, viz. *Polygonatum multiflorum*, *Yucca* sp., *Iris sibirica* var. *orientalis*, *Antirrhinum majus*, *Digitalis purpurea*, and *Pinus densiflora* did not have any influence upon the ovarian development.

After two or three days the stigma was blackened, being perhaps affected by the pollens applied.

The above experiments show that the pollinia of several orchids exert more or less a developmental stimulus upon the ovary, in case they produce pollen-tubes. However, the developmental feature of the pollen-tubes being variable according to the species used, the promotion of the ovarian development presents a certain difference in degree. As a whole, the orchids used in the present experiment do not exhibit so conspicuous an action as *Bletia*.

As to the nature of the promoting action of the *Bletia*-pollinium upon the ovarian development of *Gastrodia* I am not yet in a position to propose a definite view. However, when we refer to STRASBURGER's ('86) conclusion, that the developmental stimulus of the foreign pollens upon the ovary of certain orchids is exerted only as long as the pollen-tubes derived are alive and only within the extent they spread, and when we take into consideration the effect of the pollens of other orchids, which have been mentioned above, we may come to the view that the prominent effect of the *Bletia*-pollinium is probably assignable to a luxuriant development of the pollen-tubes entangled in the ovarian cavity, and to their long durability. The massive development of the tubes is so prominent that we can recognize them with the naked eye as a bundle of fine silk fibres, when they pass through the conducting canal of the stigma, and the durability is so long that they remain yet active when the fruit maturation is over—even about 30 days after germination¹. As regards the behaviour towards the invading tissue I would like to compare the pollen-tubes under such conditions to parasitic insects or fungi, which produce characteristic galls upon their host plants, owing, as generally accepted, to their incessant exertion of stimulus. As to a weaker action of the pollinia of other orchids in this respect, it may

1. The pollinium of *Bletia hyacinthina*, when applied to its own stigma, soon produces the pollen-tubes, which traverse a long gynostemium to reach the ovarian cavity. After a week they are found entangled among ovules, when the ovary swells a little and the ovules attain the archesporium stage. After a fortnight the ovules do not yet develop the embryo-sac. After about a month the ovary swells somewhat prominently and the ovules take on an oblong form with full-grown integument. Probably they are at the fertilization stage. The full size of the fruit is attained after three months and its maturation after four months (in October).

be probable that their affinity with *Gastrodia* is not so intimate as exhibited by *Bletia*, or as to allow of their developing the tubes massively and vigorously. In fact the maximal size of the fruit developed under their action is smaller than under the action of the *Bletia*-pollinium. Such a small fruit may be obtained under the action of the latter pollinium at deferred pollination. In this case we observe that the pollen-tubes are less numerous and less vigorous than usual.

IX. General Considerations on the Results of Experiments.

The results of the experiments recorded in the foregoing have, taken altogether, a bearing upon many problems in a wide field of research. In taking a general view over them, it will be impossible to consider every phase of the subject, and in the following only the more important matters of general interest will be presented.

I. ACTION OF THE POLLEN OR POLLEN-TUBE ON THE OVULAR AND OVARIAL DEVELOPMENT.

OVULE:—As already remarked, it has been known as a wide-spread character of the Orchidaceae that at the stage of pollination the development of the ovule is yet far from being ready for fertilization. Therefore, under ordinary conditions, the ovule is to proceed for further development while the pollen applied to the stigma germinates and sends out the pollen-tube into the ovarian cavity. The delay in the ovular development is often very striking, as can be seen, for instance, from the statement of SHARP ('12) who has investigated the embryo-sacs of several different genera of the Orchidaceae, "In most of the species here reported the pollen tubes are found growing among the ovules before the prophase of the reduction division in the megaspore mother cell." I have found quite the same thing in *Bletia*. As evidenced by the pollination experiments it is generally believed that the pollen-tube acts as stimulant for inducing the further development of the ovules. HILDEBRAND ('63) first confirms it. GUIGNARD ('86) shows that the pollen-tube exerts a stimulus on the ovules without coming in direct contact with them. As enforcing this fact, several authors, as already cited, succeeded

in promoting the ovular development in orchids by the action of the pollen from other species, genera, or families, which is of course incapable of fertilization.

As recapitulated by TISCHLER ('12, p. 65), the delay of the ovular development is reported in several other plants (*Betula*, *Alnus*, *Carpinus*, *Fagus*, *Corylus*, *Quercus*, *Hamamelis virginiana*, *Platanus* sp., *Colehium autumnale*, *Magnolia Yulan*, *Fraxinus excelsior*, *Forsythia suspensa*, *Coffea liberica*, etc). VELSER ('12) confirms the same thing in *Akebia quinata*. In all these plants it has been considered as most probable that the pollen-tubes come into play for inducing the development of the ovule ready for fertilization, as already accepted by GOEBEL ('01, p. 793). The general acceptance of this statement is now untenable. For, the study of WOLPERT ('10, p. 57) on *Alnus* brings forth the result that a normal development of the embryo-sac takes place in unpollinated flowers. FABER ('12), however, comes to contrary results in *Coffea*, finding the ovule to remain at the stage of the megaspore mother-cell if pollination is prevented. So that it is certain, that in some of the above named plants pollination is necessary and in others unnecessary upon the subject under consideration.

In *Gastrodia* the ovule at the flowering stage shows the archesporium nucleus to be at the postsynapsis stage of the heterotype mitosis (Table II). Hence the ovular development advances further than in other orchids studied by SHARP ('12), in which the same stage is attained at the time the pollen-tubes are found distributed among the ovules, and by PAGE ('07) (*Cypripedium*), who finds the archesporium nucleus to be at midsynapsis at the time the flower is at full bloom. Still, it is true that *Gastrodia* accords in the delay of the ovular development with other orchids, suggesting an agreement in the subsequent development. However, the comparative study on the pollinated and unpollinated flowers brings forth the result that the delayed development of the ovule is not an indication for the need of pollination in ensuring the development of the embryo-sac. Indeed, in either the pollinated or the unpollinated case the ovule is able to attain to the fertilization stage in the same interval, generally 4 days after bloom.

As regards the subsequent development of the ovule since the completion of the embryo-sac, the promoting action of the pollen-tube is clearly shown

by the crossing experiments with foreign pollen (Exp. 12). But it must be remembered that in *Gastrodia* the action of the pollen-tube is by no means absolutely necessary for the ovular development, because even the unpollinated flower is able to produce more or less numbers of the embryoless seeds. Hence, it may be considered that the action of the pollen-tube in question results in an increase in the amount of such seed. In this case, however, we see that the number of the seed-forming ovules varies on the one hand according to the vigour and the number of the pollen-tubes developing within the ovarial cavity, and on the other according to the available quantity of food for the growing ovules. These facts appear to show that the action of the pollen-tube may be understood as giving rise to the accumulation of the food materials within the ovary and as bringing the ovules to a better nutritive condition.

On comparing the effect of pollination of old flowers with the *Gastrodia* and the *Bletia*-pollinium (Exp. 7 and 16), we find that the ovules becoming so old as to be incapable of fertilization lose the power of response to the growth stimulus exerted by the proper or foreign pollen-tubes. As the unsuccess of fertilization in such ovules may be assigned to an internal condition of the embryo-sac, under which the egg-apparatus is unable to attract the pollen-tube, I am inclined to think that the same condition renders also the sac incapable of reacting to the growth stimulus of the tube. According to this view, the promoted growth of the ovule, due, as is assumed, to the action of the pollen-tube, is interpreted as a manifestation of the reaction of the embryo-sac to a certain stimulus exerted by the tube. The stimulus impinged upon the embryo-sac brings about the retension of its activities longer than otherwise, on account of which the nutrition of the sporophytic tissue of the ovule can be maintained so long as to ensure its full development into the seed-coat.

OVARY :—In orchids the pollen-tube is considered by several authors as exerting a developmental stimulus upon the ovary. HILDEBRAND ('63, p. 337) has shown that the pollen-tube, whatever portion of the ovary it comes in contact with, may cause the promotion of growth throughout the ovary. According to GUIGNARD ('86), the ovary is stimulated to growth by the pollen-tube, already at the time the gynostemium is completely penetrated by it,

affording an evidence for the view that the action of the tube comes into play without its direct contact with the ovary itself. FITTING ('09, p. 68) regards it probable that a swelling of the ovary may be caused by the action of ungerminated pollens, by a substance present outside them. STRASBURGER ('86) reports that a swelling of the ovary takes place prominently at the portion, with which the tubes of the foreign pollen come in contact, in lesser degree at the remoter portions. The effect of pollination, so far reviewed, is related to the prefertilization stage. At this stage the ovarial development accompanies the ovular development. It is, therefore, not conclusive whether the promotion of the ovarial development is due to the direct action of the tube, or if it is an accompanied phenomenon to the promoted development of the ovule, which is believed to be caused by the action of the tube.

It is almost general that the size of the fruit depends largely on the number of the ovules that are fertilized and develop into seeds, and of interest is the fact that at the side where the seed formation is depressed the fruit is less thickened (MÜLLER THURGAU, '08; EWERT, '06, '08; MASSART, '02). This shows clearly the dependency of the ovarial development on the ovular development. In the aitionomic parthenocarpy the fruit can grow up to the normal size, but as FITTING ('09 a, p. 202) already noticed, the ovules do not remain in most cases undeveloped, "entweder sind sie nach geringer Volumzunahme geschrumpft, oder sie sind stark gewachsen und die Samenschale ist in geringerer oder grösserer Vollkommenheit mit ihren anatomischen Eigentümlichkeiten ausgebildet worden" (cf. TISCHLER, '12). In the pollinated flower a strong development of fruit independent of a larger number of seed was reported by v. TSCHERMAK ('02) in the pollination experiments in *Cheiranthus*. He found that the fruit produced by xenogamy is larger than that produced autogamously (p. 15), in spite of the smaller amount of the seed developed. But, as he remarked, this is not constant.

The instance of the development of the fruit-wall without the accompanying ovular development can be found in parthenocarpy (see TISCHLER '12). But here no action of the pollen-tube is concerned. Regarding a similar case of development in connection with the action of the pollen-tube our knowledge is yet inadequate.

An attempt has been made in *Gastrodia* to elucidate this point. The related data brought forward by several experiments are as follows:

1. Its own pollinium gives rise to the capsule, whose size corresponds to the number of the seed.

2. Old flowers pollinated with their own pollinia produce capsules exceeding only a little in size, and also in amount of the seed contained, those obtained from the unpollinated flowers (Exp. 7).

3. Pollinated with their own pollinia, the flowers which have been previously pollinated by *Bletia* produce full-sized capsules, in which the seed, embryogenic and embryoless, is smaller in amount than in the normal capsule (Exp. 17-19).

4. The pollen-tubes do not promote the ovarial development, if all the ovules are injured at the time of pollination (Exp. 11).

5. The *Bletia*-pollinium introduced into the ovary results in the formation of a normal-sized capsule, though the amount of the seed contained may be smaller than otherwise (Exp. 15).

6. Old flowers pollinated by *Bletia* give rise to capsules much smaller than the normal ones; in this case the amount of the seed is proportionally decreased (Exp. 16).

Thus the promotion of the ovarial development in the pollinated flowers always accompanies the seed formation of a larger or lesser number of ovules. Pollination into the ovary with foreign pollen (case 5) appears to show that the ovarial growth is proportional to the vigour of the pollen-tube rather than to the amount of the seed. It happens, though seldom, that the capsule obtained from the pollinated flower under certain conditions is only a little larger than that from the unpollinated flower, in spite of a nearly equal amount of the seed contained. However, these facts are not sufficient to justify the belief that the ovary may be promoted in development by the action of the pollen-tube independent of the ovular development. Referring to the case 4 above mentioned, the ovary appears to depend on the ovule for its growth.

2. CORRELATION BETWEEN THE DEVELOPMENTAL PHASES DURING THE FRUIT FORMATION.

The normal fruit formation in the fertilized angiospermous flowers involves

several developmental changes. Confining ourselves to the ovary, they occur in association among themselves, under ordinary conditions, in the ovarian wall for the fruit, in the sporophytic tissue of the ovule for the seed, and in the embryo-sac for the embryo and endosperm. As to how they relate to each other, we must attempt first of all to analyse by experimental studies the entire phase of the fruit formation into its components. In *Gastrodia* the development of the endosperm is quite suppressed, and the chief components to be considered are attached to the ovarian wall, the ovular tissue, and the embryo. The dependency or independency of their development on each other, as far as ascertained by experiments, will be seen in the following:

1. Fruit, seed, and embryo all normal: when normally fertilized and subjected to normal conditions.

2. Fruit and seed quite normal, but embryoless: when pollinated with *Bletia*-pollinium under normal conditions.

3. Small fruit and normal but embryoless seed: when unpollinated; the number of seed is accordingly diminished.

4. Imperfect or almost no fruit, but normal seed with embryo: when the normally fertilized flower is separated from its nutritive connection.

5. Imperfect or almost no fruit, and nearly normal but embryoless seed: when the unpollinated flower is parted from its nutritive connection; the number of seed is exceedingly diminished.

6. Imperfect or almost no fruit and seed, but almost normal embryo: when the fertilized flower is subjected to an extremely unfavourable condition of nutrition. In this case the typical integument is quite suppressed in development and the ovular tissue developed previous to the fertilization stage partakes the formation of the imperfect seed-coat.

The other combinations, viz. the normal fruit without the seed and embryo, and the fruit with the embryo but without the seed, are not producible.

From the above we see, that the embryo does not require during its development the accompaniment of the normal development of the ovarian wall and the sporophytic ovular tissue and that the seed-coat alone can develop completely, independent of the formation of the embryo, or of the normal development of the fruit-wall. But it must be remembered that a

nutritive condition which renders the development of the fruit-wall unfavourable may bring about a small amount of embryoless seed.

In the process of fructification the embryo is placed in the first rank for development; if the nutritive condition is favourable, it accompanies the development of the seed-coat and fruit-wall; if not, only the latter portions are in high degree retarded in development. A similar relation may exist between the fruit-wall and the embryoless seed; under the condition which induces most ovules to develop into embryoless seeds the fruit-wall develops most vigorously; under an insufficient supply of nutritive substances the number of the seed-forming ovules is diminished, and in this case the fruit-wall is sacrificed for development; in the extreme case of an insufficient nutrition both the fruit-wall and a larger number of ovules are suppressed in development, thereby supplying limited nutritive material to a few ovules, enabling them to form seed. It may be ascribed to such mutual relations, that in *Gastrodia* the normal-sized fruit without the seed and embryo, or with the embryo but without the seed, cannot be obtained. The development of the fruit-wall alone under entire suppression of the ovular development is found in some instances of the habitual parthenocarpy.

3. PARTHENOCARPY.

In TISCHLER's careful critical enumeration of parthenocarpic plants mention has not been made of the Orchidaceae. However, as he says (p. 62), FOCKE ('81, p. 480) reports the formation of an externally well-developed but seedless fruit in some bastard orchids. TISCHLER (p. 65) further states, "Die Möglichkeit eines relativ leichten „Fruchtungsvermögens“ wird von GÄRTNER für die Orchideen ganz allgemein, . . . , angegeben." According to TREUB ('83), the ovary of *Liparis latifolia* undergoes a fruit-like swelling when attacked by an insect larva (FITTING, '09a, p. 235, mentions that a similar case is given by FORBES in *Calanthe*). From these facts it appears that the parthenocarpy in the Orchidaceae is known since older times, though a detailed account of its true nature has not been given. As *Gastrodia* offers a good example of parthenocarpy among orchids, I shall here record a somewhat detailed account of this mode of the fruit formation.

In *Gastrodia* parthenocarpy cannot be said to be habitual, since its male and

female gametophytes show a quite normal development, ensuring fertilization and the normal fruit formation. But as an interesting fact, unpollinated flowers are by no means incapable of fruit formation. Their ovaries are always persistent as long as those of fertilized flowers, or rather still longer, meanwhile the necessary developmental processes are going on. On account of the smaller size the resulting fruit cannot be placed in the same rank as that derived from the fertilized flower, but in general features the ovarial wall acquires the nature of the wall of the typical fruit. The fruit nature is further enhanced by the development of some ovules into embryoless seeds, having a seed-coat of the usual structure. In the seed-producing species of banana d'ANGRÉMOND ('14) has recently confirmed that the ovary of an unpollinated flower persists on the axis, without showing any growth, but acquiring the nature of a mature fruit a little earlier than the normal fruit. Whether it can be looked upon as an instance of parthenocarpy is doubtful, as, unlike *Gastrodia*, it does not produce seed.

In studying parthenocarpy our attention is at present drawn to its causal relation. The formation of the embryoless fruit without agency of any external factor is denoted as the vegetative (according to WINKLER) or autonomic (according to FITTING) parthenocarpy. We distinguish from it the stimulative (WINKLER) or aitionomic (FITTING) parthenocarpy, as being induced by the stimulus of pollen or any other agent. As will be seen in TISCHLER's recapitulation, numerous instances of parthenocarpy hitherto recorded were not precisely investigated, and but little attention has been paid as to whether both autonomic and aitionomic parthenocarpy is possible or not in one and the same plant. Recently d'ANGREMOND ('14, p. 68) made an experiment on this subject with banana. He pollinated the flower of a habitually autonomic parthenocarpic form with the pollen of other seed-producing forms, but he could not ascertain a developmental stimulus of the pollen at all, as both unpollinated and pollinated flowers yielded quite similar-sized fruits. The case is different in *Gastrodia*. The parthenocarpy is induced both autonomically and aitionomically, but the size of the resulting fruit presents a great divergence: the aitionomic fruit derived by crossing *Blétia* attains to a size as large as the normal one, while the autonomic fruit is remarkably smaller.

Subjecting flowers to different nutritive conditions we may obtain the

parthenocarpic fruits in various sizes. Variation in size is by no means a strange feature in normal fruits, as already experienced by gardeners. The point of interest in this connection, as far as it concerns parthenocarpy, is that under the same external condition the fruit produced autonomically is smaller than that produced aitionomically, even under the most favourable condition.

As to how both kinds of the parthenocarpic fruit display such a difference, further extended researches would reveal perhaps interesting facts. As a tentative view I can express it at present as follows: in the autonomic case the nutritive material, at the expense of which the ovary can grow, is accumulated passively, so that the presence of a surplus amount of it in the entire plant body would favour the formation of a larger fruit than otherwise (Exp. 3); but in the aitionomic case it is accumulated actively; in other words, a stimulus upon the ovary acts as withdrawing the nutritive material in the ovary from the store house; this may submit the ovary to better nutrition.

As regards the parthenocarpic development by the foreign pollen two points may be worthy of consideration. First, the size of the resulting fruit may depend on the intensity of the stimulus. This is evidenced by the experiment with the *Bletia*-pollinium: pollinated the day of bloom, the pollinium sends out massive tubes, leading the fruit to maximal growth, but the delayed pollination brings about a feebler development of the tube, perhaps owing to a certain modified condition of the stigma, and consequently smaller fruits result. Further, the pollinia of other orchids yield smaller fruits than the *Bletia*-pollinium, in conformity with the feeble development of the pollen-tubes. Secondly, it may be most probable that the size of the fruit correlates with the duration of the stimulus acted upon. The product of the normal-sized fruit by crossing *Bletia* appears to be due to the longevity of activity of the pollen-tube, remaining alive and vigorous far beyond the period of maturation of the fruit, and thus exerting the stimulus unceasingly upon the ovules and ovary throughout the interval of their complete development. Whether this assumption is correct or not, will be decided by the results of experiments with *Gastrodia* or other plants crossed by certain germinative foreign pollens, which are able to promote ovarial growth, and whose tubes are short-lived or long-lived with respect to the maturation period of

the fruit. That the prosecution of such experiments is highly desirable will be conceived, when we look over the results of investigations by previous authors on the promoting action of several agents upon the ovarial growth. To give instances from the Orchidaceae, HILDEBRAND's and STRASBURGER's cross-experiments with foreign pollens have shown the swelling of the ovarial wall in so far as the pollen-tube came in contact with it. According to STRASBURGER ('86), the promoted growth of the ovary of *Orchis Moris* and *O. mascula*, when crossed by *Fritillaria*, is observable only as long as the tube remains alive. His failure of getting the mature fruit appears to me due to an earlier cessation of the stimulating action with respect to the interval needed for maturing the fruit. As HILDEBRAND (cited from GUIGNARD, '86) reports that fertilization takes place in *Orchis mascula* 28 days after pollination with its own pollinium, it is likely that in STRASBURGER's experiment the combination of short-lived pollen-tubes and a slowly developing ovary has been made.

In this connection FITTING's ('09 b, '10) very elaborate and highly suggestive experiments on orchid flowers are of special interest. He analyses the action involved in pollination into that caused by the pollinium itself and that caused by the pollen-tube. According to him, the chemical substances attached to the surface of the pollen-grains exert a stimulating action upon several floral organs and, in certain species, may cause an exceedingly slight swelling of the ovary. He further confirms that the stimulus of the pollen-tube inducing the growth of the ovary is different from that exerted by the extract of the pollinium when applied to the stigma, and that its effect, whether the pollen taken is from other genera or families, is more remarkable than that of the pollen extract. Agreeing with HILDEBRAND and STRASBURGER, the action of the pollen-tube is exhibited when it penetrates into the ovary. As to whether the stimulus exerted by the tube is of a chemical nature or not, he leaves for a future investigation: "Der Einfluss der Pollenschläuche bleibt ein Problem für sich, das besonderer Untersuchungen bedarf" ('10, p. 258). As far as observed in *Gastrodia*, we are led to the view that the ovarial development is correlated with the embryogenic development of the ovules when the tube of its own pollinium is concerned, but when it is induced by the foreign pollen-tube, it is likely comparable to

the gall formation by the action of fungi or insects. So that, though the kind of the stimulus is unknown, whether chemical or mechanical, we may ascribe the resulting effect to an incessant stimulus of sufficient intensity. This is another problem, I believe, attached to the action of the pollen-tube and to be answered by further extended experiments.

On several fruit trees EWERT ('07, '08, '09) and MÜLLER THURGAU ('08), after carrying out repeated experiments, succeeded in obtaining facultative parthenocarpic fruit. As for its aetiology both authors arrived at the same conclusion, that the fruit formation in question correlates with the amount of nutritive substances in the mother plants, which can be supplied to the fruit. It was evidenced by the fact that, when in all the flowers of an entire individual plant pollination was prevented or when the unpollinated flowers were situated beyond the region of ringing ("Ringelung") of a branch, they were disposed to effect parthenocarpy. Of dependency in the growth of parthenocarpic fruit of *Gastrodia* on the nutritive condition I agree with EWERT and MÜLLER THURGAU'S view. But as regards the causal relation between the nutritive condition and parthenocarpy, *Gastrodia* does not accord with the plants studied by the two authors. For, in *Gastrodia* the unpollinated flowers are accustomed to produce the capsule, though small, under any condition, and this inherent character is expressed more pronouncedly when the nutritive condition is more favourable.

In the strict sense of the word, parthenocarpy is concerned with the development of the ovarial wall into fruit. But as we have pointed out before, there exists an intermediate relation between the ovular and ovarial development. TISCHLER'S ('12) cytological studies on the parthenocarpic fruits in Angiosperms will clearly show how variable is the relation between the development involved in the ovule and that of the ovary: (1) in some plants the endosperm and seed-coat are formed; (2) in some only the seed-coat is formed; and (3) in many others the ovules disorganize entirely, sometimes previous to the completion of the embryo-sac or immediately after. *Gastrodia* affords an example of the second class, and in this case my experiments brought forth the result that the parthenocarpic development of the ovary is dependent upon the ovular development and that the amount of the seed yielded governs in more or less degree the size of the capsule. Such relation

between the ovule and ovary has been ascertained in the instances of parthenocarpy, as demonstrated by EWERT ('09, p. 837), "dass das bessere Fruchtungsvermögen der Birne zum Teil auf der leichten Entwicklungsfähigkeit der unbefruchteten Eiknospe beruht."

4. POLYEMBRYONY.

A frequent derivation of the embryo from a synergid is known in several plants (cf. ERNST, '88; COULTER and CHAMBERLAIN, '03). In parthenogenetic plants it is evidently produced asexually (for instance, *Alchemilla*, MURBECK, '02; *Burmannia*, ERNST and BERNARD, '12), but in ordinary plants, in which the egg needs fertilization on developing into the embryo, the synergid embryo is also considered as the product of the sexual act, without any convincing proof in all the cases. As to under what conditions the development of such adventitious embryos is induced, nothing definite has hitherto been reported.

In the Orchidaceae it has been reported by STRASBURGER in 1878 that *Gymnadenia conopsea* often produces two embryos in a single embryo-sac, probably one of them being derived from a synergid, according to COULTER and CHAMBERLAIN ('03, p. 217), either apogamously or by fertilization.

In *Gastrodia* polyembryony is exhibited exclusively in the fertilized ovule. This is evidenced by the presence of the pollen-tube within the sac, or, if not, of a hyaline mass, originated from the disorganized tube and synergid. This fact prevents our concluding that polyembryony might have connection with the parthenogenetic development of the egg. In the whole aspect it is clear that one of the two embryos is derived from the fertilized egg. As to the other embryo, its position and general form point to its origin as a synergid (Fig. 108). Whether it has been derived by fertilization or not, however, is difficult to state with certainty, as neither the fusing stage of the gamete nuclei¹ nor the chromosome number of the embryo could be followed out. Probably fertilization took place. At first both proembryos assume quite the same shape (Fig. 108); afterwards one of the two suspensors elongates more than the other and makes the two embryos arrange in longitudinal

1. Only once I observed a synergid which gave the appearance as if invaded by a male nucleus.

order (Fig. 109). They both undergo further development until maturation.

The occurrence of the synergid embryo is exceedingly rare in flowers pollinated at the due time. Among about 25 young fruits carefully examined only 3 were found to contain the polyembryonic seed, but its number in a single fruit did not exceed more than one per cent. of the seeds. On the other hand, it occurred almost regularly when pollination was delayed for 3-4 days (Experiment 7). However, even in this case the polyembryonic seeds were comparatively few in number; at most they amounted to 5 per cent., while usually only 1-2 per cent. of the seeds of a single fruit contained two embryos. From this fact I conclude that the delay of pollination tends to give rise to polyembryony¹.

As regards the determining condition of polyembryony the experimental data do not yet offer sufficient evidence for the advancement of any acceptable view. Provisionally I may be allowed to make the following consideration:

We have already shown that in flowers pollinated on the day of bloom the egg-apparatus becomes organized, just ready for fertilization after 3-4 days. When pollination is delayed for 3-4 days, fertilization must be postponed for the same interval. It may be assumed that the egg apparatus undergoes a certain modification in the act of fertilization during this interval. At what internal state in this case the synergids just at the period of entry of the pollen-tube are, we are scarcely able to know. However, about the mechanism which causes one of the synergids to develop into an embryo, it may be presumed that its nucleus becomes simply incapable of slipping out from the cell to migrate down for approaching the pole nucleus, and hence it is in the state to receive the male nucleus at its proper position. In *Gastrodia*, differing from other plants which develop the usual 8-nucleate sac, the synergid nucleus is endowed with the property to fuse with both the male and the pole nuclei, so that the supposed condition under

1. This statement is based on the observation of the material obtained in 1912-1914. To furnish reliable material for comparison one set of flowers was pollinated on the day of bloom and the other set in the same inflorescence was pollinated on the same day when opened 3-4 days previous to those of the former set, or 3-4 days later when opened on the same day as those of the former.

which the synergid nucleus loses the power of motion may be looked upon as the first impetus to the development of the synergid embryo. How this power is lost, is a point needing further consideration. As a fact, however, we may state that the power of motion of the synergid nucleus is first displayed, in normal condition, when the pollen-tube liberates the male nuclei. If the entry of the tube does not take place, the synergid remains intact for a certain period and then begins to degenerate with the nucleus enclosed. This fact lends support to the view that the synergid becomes after a certain period incapable of shedding its nucleus, even though the pollen-tube enters the embryo-sac. It may also be conjectured that the synergid nucleus, while attaining this stage, is becoming inactive for fusion with the male nucleus. The chance of liberation of the male nucleus just at the critical period, at which the synergid nucleus becomes incapable of slipping out from the cell but yet retains the fusing activity, would be exceedingly rare. That in fact only a small percentage of the ovules produce the synergid embryo, is thus explained.

The above assumption may also be applicable to explain the still rarer occurrence of the synergid embryo in flowers pollinated on the day of bloom. Although the majority of the ovules completes the embryo-sac nearly simultaneously, just at the time the pollen-tube is ready for fertilization, that is, 3-4 days after pollination, there may occur, as already mentioned, a comparatively few ovules that advance further in development than others (see Table II) and complete the embryo-sacs 2-3 days earlier. These early completed embryo-sacs must wait for the pollen-tube, as do most sacs in the case of delayed pollination.

In the polyembryogenic sac we find a single nucleus corresponding to the endosperm nucleus, even when the embryos attain to the multinucleate stage (Fig. 109). Normally it is the product of triple fusion, but in the present case it is really the pole nucleus itself, since here the two nuclei for triple fusion—the synergid and the male nuclei—behave otherwise, and the other synergid nucleus, which is to degenerate in the usual case soon after the entry of the pollen-tube, does not seem to fuse with the pole nucleus. In consequence, the polyembryogenic sac cannot produce the true endosperm nucleus. It is interesting that here the pole nucleus can remain intact even

6 days after pollination, as far as observed, in other words, till the period when the embryos attain a multicellular stage: certainly it would behave quite similar to the true endosperm nucleus, if it were to take some part in the activities of the embryo-sac.

It may be remarked that the behaviour of the synergid in the 4-nucleate embryo-sac, as observed in *Cypripedium* and *Gastrodia*, is different from that in the usual 8-nucleate embryo-sac, especially as regards the fusing activity. Hence it would appear that the hypothesis advanced, relating to the condition of the development of the synergid embryo in the case of the 4-nucleate sac, permits no application on the same phenomenon often observed in the 8-nucleate sac.

5. THE EMBRYO-SAC IN THE OVULE DEVELOPING INTO THE EMBRYOLESS SEED.

It has been shown that almost all ovules in a flower, whether pollinated or not, go to complete the embryo-sac after certain days of bloom. If fertilization is prevented, most sacs disorganize ultimately. This process begins with the independent obliteration of cells composing the egg-apparatus or of the pole nucleus; at first the synergids obliterate themselves in some sacs, or the pole nucleus in others. The obliteration of cells or nuclei in the sac is a phenomenon often observed, independent of pollination or fertilization, as it takes place soon after the completion of the embryo-sac or even a little earlier. It brings about the shrinkage of the sac and the accompanying shrinkage of the ovule itself, arresting its subsequent development. On the other hand, in the surviving ovules, which continue the further growth, we always find intact sacs. Accompanying the growth of the ovule the egg-cell often enlarges with an increase of plasmic contents, and the pole nucleus is found to have a pronounced reticular structure and sometimes 2-3 nucleoli, showing a high activity, while the synergids appear somewhat shrunken. As far as ascertained in microtome sections, such a character of the sac is maintained for 12 days after bloom, while the ovular development is yet far from completion of seed. After 14 days, when the surviving ovule develops into a seed still immature, we can find the egg-cell and pole nucleus still alive in the spacious cavity of the sac.

From this fact it is almost certain that the capability of the unfertilized ovules of developing into embryoless seeds is intimately connected with the retaining activity of the embryo-sac. As to the causal relation of the ovular development and the survival of the embryo-sac, one might infer that the condition which causes the ovule to survive and to continue its further growth may bring about the survival of the sac. In d'ANGREMOND's ('14) study on banana the growth of the ovules is observed after the obliteration of the embryo-sac at any developmental stage. As far as my observations extend, there cannot be found any ovules that are able to grow without the intact sac, and, as mentioned above, the first sign of obliteration of the ovule occurs in the sac. This would show that the growth of the unfertilized ovule depends on the condition which enables the sac to remain alive.

In a mature parthenocarpic fruit we find ripe seed of various degrees in size. This will show the nutritive variations among the growing ovules in so marked a degree, as we are unable to find in the fertilized ovules under normal conditions. The variation is emphasized when the ovules are subjected to an insufficient nutrition, equally in fertilized and unpollinated flowers. Thus in the case of the aitionomic parthenocarpy the most favourable condition of nutrition renders possible almost all embryoless seeds to attain the normal size. When the nutritive condition is unfavourable, the number of seed is much diminished, the less the number, the more unfavourable the condition. In the latter case the struggle for existence occurs among the seed-forming ovules, some being unsuccessful in forming seed, some developing smaller seeds, while the most vigorous ovules succeed in the formation of full-sized seeds. All the seeds in the same capsule, whether smaller- or full-sized, come to maturity at the same period. They have been derived from ovules containing the embryo-sacs all alive, and the nutritive activity of the embryo-sac, or rather, in my opinion, of the pole nucleus, would have been of variable intensities, with which the size of the resulting seed may correlate.

In fertilized flowers we always find a certain number of unfertilized ovules, even under the most favourable condition. Also the parthenocarpic fruits, developed under favourable conditions, contain a certain number of degenerating ovules incapable of seed formation. Such a feature of the ovular

development would be of special interest, if we take into consideration the fact that the chromosome reduction is omitted in some ovules. It is almost certain that the diploid egg cannot effect fertilization or derive the parthenogenetic embryo. In consequence, the ovule possessing the diploid egg is not able to develop a seed in the normally pollinated flower. As an analogous case VEITCH ('88) reports in *Cattleya labiata* var. *Morsiae* the regular occurrence of a certain number of sterile ovules (about one half). In the case of the unpollinated flower it may be supposed that the survival of many ovules is assigned to the omission of the chromosome reduction, while the obliteration of other ovules is connected with its occurrence. Theoretically this may be highly probable. If we accept this assumption, it should be possible that the seed-forming ovules in the unpollinated flower represent the degenerating ones in the pollinated flower. Unfortunately, convincing evidence for this relation is not obtained from observations, since the number of the ovule to derive the embryoless seed in the unpollinated flower and to degenerate in the pollinated one presents individual variations. As far as my observations go, I am rather inclined to the view that in the pollinated flower the ovules having the diploid egg are all obliterated, and in the unpollinated flower the embryoless seeds may originate not only from these ovules but from those having the haploid egg as well. As we have already explained, it is sufficient that for seed-formation the embryo-sac of the unfertilized ovule remains intact for two weeks or a little longer. As most embryo-sacs in the unpollinated flower are yet alive after a week of bloom, the surviving and degenerating ovules appear, as shown by the experiments and observations, to be differentiated at about this period or one to two days later. Among these surviving ovules the embryo-sac containing the haploid egg is found, maintaining its activity for a few days more (see Experiment 3). In the fruit produced by crossing *Bletia* the amount of seeds is often too large to assume them all to have been derived from the ovules having the diploid egg. These facts give evidence for the view that the ovule possessing either the haploid or diploid egg is able to develop into the embryoless seed.

6. PARTHENOGENESIS.

In Angiosperms the habitual parthenogenesis (apogamy according to

STRASBURGER) usually accompanies more or less disturbance of the microspore development (WINKLER, '08, p. 384; SHIBATA and MIYAKE, '08; OSAWA, '13). As exceptions, however, *Thalictrum purpurascens*, *Hieracium aurantiacum* (apospory), and *Alamosco* (PACE, '13) produce normal pollen-grains. In connection with this the female sex often undergoes no complete adaptation to apomixis. According to OVERTON ('02, '04), *Thalictrum purpurascens* develops both haploid and diploid eggs, and by pollination only the haploid egg is fertilized to produce the embryo, while the prevention of pollination gives rise to the parthenogenetic development of the diploid egg.

It has been pointed out that in *Gastrodia* the pollinium functions quite normally, but no less numbers of the embryo-sac are derived from megaspores having the diploid number of chromosomes. In this respect the gametophytic development in *Gastrodia* presents quite the same peculiarity as in *Thalictrum*. We may now presume *a priori* the possibility of both apomictic and amphimictic development of the embryo. But my observations and experiments brought forth a contrary result. In nature the embryo development is exclusively amphimictic. If pollination is prevented, no embryo develops, though the sporophytic tissue of the ovule may undergo the normal development. It may be conceived that some of the embryo-sacs are most probably ready, as regards the nuclear feature, for the somatic parthenogenesis (according to WINKLER), but the accommodation of other characters does not advance so far as to realise it.

In the somatic parthenogenetic fruit the development of the diploid egg into the embryo accompanies the complete development of the sporophytic tissue of the ovule. This points to a close affinity between parthenocarpy and parthenogenesis; indeed parthenogenesis belongs to the category of parthenocarpy, as maintained already by NOLL, showing the embryogenic parthenocarpy. *Wickstroemia* investigated by WINKLER ('05) is a habitually parthenogenetic plant, but according to him, it appears incapable of producing a typical parthenocarpic fruit, though a slight swelling of the ovary may take place without accompanying embryo formation. A somewhat reciprocal relation is shown in *Gastrodia*. Here parthenocarpy approaches parthenogenesis very closely in producing the perfect fruit-wall and seed-coat. It is remarkable that, though the embryo is wanting, the undivided egg is

able meanwhile to increase in size previous to its obliteration. A tendency towards the parthenogenetic development being thus conceivable, attention has been paid to the question whether multiplication of cells in the egg among the seed-forming ovules might occur. After a long search I was able to obtain a confirmatory fact. In the ovaries 6 days after pollination with the *Bletia*-pollinium, and also 11-12 days after bloom in the unpollinated stock (Experiment 3), I could find the egg nuclei in division. It must be remembered that the *Bletia*-pollinium is unable to fertilize, and the division must have taken place in the unfertilized egg. The eggs in division were exceedingly few in number and only four slides showed the karyokinetic figures (Figs. 110-113), the most advanced stage I examined giving two daughter nuclei.

Of several karyokinetic figures only three offered a very distinct view of chromosomes. The existence of the diploid egg indicated that the division was partaken of by such eggs, exhibiting, just like *Thalictrum purpurascens*, the somatic parthenogenesis. But in fact a careful counting gave 8 or 9 chromosomes, exactly the haploid number (Figs. 110, 111). Although the material was scanty, it was sufficient and reliable enough for estimating the exact number of chromosomes with accuracy. Notwithstanding, I do not like to preclude the idea of the possibility of the parthenogenetic division in the diploid egg, but the evidence at hand leads us to conclude that the haploid egg alone is able to proceed for the parthenogenetic development. This concerns the true parthenogenesis (STRASEBURGER) or the generative parthenogenesis (WINKLER) caused facultatively.

Generally, the parthenogenetic egg is smaller than the fertilized one, and also the karyokinetic figure is correspondingly smaller (compare Figs. 103 and 110-113). The direction of the spindle may be longitudinal, transverse, or oblique (Figs. 110, 112).

In *Monotropa uniflora* SHIBATA ('02) observed in the unpollinated flowers the survival of a certain number of the embryo-sac, producing the parthenogenetic endosperm therein. Occasionally he found the division of an enlarged egg into two. Though no account of the number of chromosomes was given, it can scarcely be doubted that the egg nucleus was haploid. In studying the parthenocarpic development in *Ficus Carica*, TISCHLER ('12, p. 19) found

one of the unfertilized eggs, usually assuming an enormous size, in nuclear divisions (deriving 132 nuclei). As he noted, evidence of the haploid nature of the egg was lacking, but he considered it highly probable, as presenting a case of the generative parthenogenesis. Thus in *Monotropa*, *Ficus*, and *Gastrodia* I find a certain similarity in the condition of the egg-cell which undergoes the parthenogenetic division; its hypertrophied appearance seems to point to its being under the condition of excessive nutrition.

The haploid egg in such an abnormal development is incapable of deriving the parthenogenetic embryo. As an interesting fact, the nuclear division does not accompany the cell division. In *Gastrodia* two eggs were found to possess two resting nuclei, which appeared to have passed far beyond the telophase stage; no limiting wall, however, was formed between. This accords with the case in *Ficus Carica*. It is interesting to note that the egg-cells of *Orchis Morio*, which is fertilized, according to STRASBURGER ('86, p. 62), by *O. fusca*, undergoes the nuclear division, but not the cell division.

Now referring to other orchids I call attention to the so-called "false hybrid" (after MILLARDET's meaning) in *Zygopetalum*. According to HURST ('89, '03), it is characterised by the fact that the crossing of *Z. Mackayi* by several other orchids produces the offspring proving to be *Zygopetalum* pure and simple. He proposes the view as an explanation of this character of the hybrid, that it shows a kind of parthenogenesis, the foreign pollen exerting stimulus upon the egg, but not effecting actual fusion between the sexual elements. As already remarked by WINKLER ('08), if parthenogenesis really occurs, the egg nucleus might be diploid. The occurrence of both haploid and diploid eggs in *Gastrodia* and the promoting action of the foreign pollen on their development would appear to give a hint for the explanation of this peculiar phenomenon in *Zygopetalum*. Also several plants commented by FOCKE ('81, p. 525) as performing "pseudogamy" would require cytological researches, in order to ascertain, whether the foreign pollen applied acts fertilizing or as stimulative upon the diploid egg for its parthenogenetic development.

With reference to the parthenogenetic problem of *Gastrodia*, the most interesting paper of PACE ('13) relating to the vegetative parthenogenesis of *Atamosco*, one of the Amaryllidaceae, should have an important bearing. The egg is ascertained to contain the diploid number of chromosomes, and on

fertilization "two nuclei come in the sac with the pollen-tube, one fusing with the two polars, the other entering the egg but never fusing with it, and finally disintegrating during the first division in the egg" (p. 390). From such a unique conduct of fertilization we infer that the entry of the pollen-tube and even of the male nuclei into the embryo-sac is not always an indication of actual fertilization. In *Gastrodia* a question arises as to the relation between the diploid egg and the pollen-tube. Supported by the fact found by PACE, it is not quite impossible to conceive that the diploid egg of *Gastrodia* might perhaps perform the parthenogenetic development, being stimulated by the pollen-tube coming into the sac. If this be true, we must infer that in *Gastrodia* two kinds of the embryo develop, one by amphimixis and the other by apomixis. However, I was not able to find any sac which was penetrated by the pollen-tube, and in which the egg was in nuclear division accompanying an unfused and disorganizing male nucleus near the egg nucleus. So that I am of the opinion, that the embryo arising from the pollinated flower is exclusively the product of the sexual act.

That the cause of the facultative parthenocarpy is concerned with the nutritive condition has been maintained by several authors, and in *Gastrodia* it has been shown that excessive nutritive materials may promote the development of the autonomic parthenocarpic fruit. Some authors maintain a similar condition of nutrition as causing the parthenogenetic development (see WINKLER, '08, p. 420), while WINKLER disagrees with this hypothesis. In *Gastrodia* an occasional nuclear division in the haploid egg and the vigorous development of the parthenocarpic fruit are of parallel occurrence: in both autonomic and aitionomic parthenocarpy the seed-forming ovules increase in number, when the nutritive condition is favourable, and in these ovules the egg is much hypertrophied. Thus the nuclear division in the haploid egg may be ascribed to an excessive nutrition operating on the egg, suggesting the existence of a certain relation between nutritive condition and facultative parthenogenesis.

7. DEVELOPMENT OF THE SPOROPHYTIC TISSUE OF THE OVULE.

The sporophytic tissue of the ovule participates in the formation of the seed-coat. Taking now this tissue as the object of consideration, we see that its full development is manifested in certain relations to other tissues concerned with the fructification, such as the ovarial wall and the embryo-sac, and further it is induced by the action of the pollen-tube or other agents, or independent of such external factors. Several cases in this respect are already found scattered in the literature, and additional cases are afforded by the present investigation. To make the matter comprehensive they may be arranged in the following manner :

I. PREFERTILIZATION STAGE. It comprises the stages of the ovular development, through which the ovule completes the embryo-sac.

A. *Autonomic*: Induced without any external stimulus (usual and parthenogenetic plants).

B. *Aitionomic*: Induced only by pollination (most of the Orchidaceae, many species of the Amentaceae, and other plants) (cf. TISCHLER, '12, p. 69).

II. POSTFERTILIZATION STAGE. It comprises the developmental stages subsequent to fertilization till the completion of the seed-coat.

A. *Embryogenic*: The formation of the seed-coat accompanies the development of the embryo inside.

a. *Autonomic*: Without pollination.

1. *Carpous*: In parthenogenetic plants under ordinary conditions.

2. *Acarpous*: No case is yet reported. If the ovule of certain parthenogenetic plants be capable of the full development under the condition similar to that, under which *Gastrodia* produces embryogenic seeds without accompanying the typical fruit formation (see below), it would afford the example.

b. *Aitionomic*: Induced by pollination.

1. *Carpous*: In usual plants under ordinary conditions.

2. *Acarpous*: *Gastrodia* affords an example, when the fertilized

flower is subjected to unfavourable conditions of nutrition; here the ovarial development is almost entirely arrested and the normal fruit is scarcely produced.

B. Sterile: The seed-coat is completed without accompanying the development of the embryo.

a. *Autonomic*: Without pollination.

1. Carpous: In autonomic parthenocarpic plants (*Gastrodia* and other plants enumerated by TISCHLER, '12).
2. Acarpous: In *Gastrodia*, when the abscised flower is left unpollinated; the amount of seed is diminished considerably.

b. *Aitonomic*: Induced by pollination or by external stimuli.

1. Carpous: In aitonomic parthenocarpic plants (*Gastrodia* pollinated by *Bletia*).
2. Acarpous: In *Gastrodia*, when the abscised flower is pollinated by *Bletia*; the amount of seed is larger than in the autonomic case.

It must be remarked that the word "acarpous" is accepted conventionally. In the strict sense, it would be absurd to conceive the existence of either the embryogenic or the sterile seed in the absence of the fruit-wall. The capsule of *Gastrodia*, differing from that of other plants, presents no remarkable histological divergence from the ovary, from which it is derived. Chiefly the enlargement of the component cells in the ovary suffices to produce the capsule, without multiplication of the cells and the thickening of their wall. The difference in size, which can be taken as a chief distinction between the seeded capsule and the ovary, is very variable according to the nutritive condition under which the capsule is developing; there may occur so small a capsule that it scarcely exceeds in size the ovary at the fertilization stage. As already described, the ovule needs after bloom a further development, in order to complete the embryo-sac, and in association with this the ovary makes a corresponding growth after bloom. Such a growth of the ovary should not be taken, in my opinion, as related to the fruit formation. For this reason I apply the term "acarpous" to the case of the seed formation, during which interval the ovarial wall does not undergo essential changes

after the fertilization stage. On the acarpous development of seed, WINKLER ('08, p. 395) remarks, "Darüber, ob auch umgekehrt normale Samenentwicklung ohne Fruchtbildung möglich ist, scheint nichts bekannt zu sein; theoretisch ist es natürlich sehr wohl denkbar, und wenn ich nicht irre, kommt es z. B. beim Wein gelegentlich vor, dass die Beere nicht zur Ausbildung kommt, obwohl sich gesunde Samen entwickeln, so dass diese dann anstatt von der Beere nur von einer dünnen Fruchthaut umhüllt werden." Of course a habitual occurrence of the seed without the fruit is highly inconceivable, but artificially it would not be quite impossible to obtain in other plants the seed with a considerable inhibition of the fruit development, as exemplified by *Gastrodia*.

From the above considerations it will be seen that several problems on the phenomena of the embryonal development are thrown into great confusion on account of the frequent omission of the reduction division. So that the views so far advanced may be largely hypothetical. Some of the problems demand further investigation under special treatment. I shall be content, if the present study may arouse interest for the experimental cytological studies on the generative sphere in Angiosperms.

X. Summary.

The results of the observations and experiments have already been recorded in proper places. Here only the chief facts will be recapitulated.

1. The flower is, in a high degree, resistible towards various kinds of treatment, and the embryonal development exhibits extreme simplicity and is completed with great rapidity. On this account *Gastrodia* offers the best-suited material for the experimental study on the embryonal development in Angiosperms.

2. The embryo-sac is completed 3-4 days after bloom, and the same interval of time is required for the pollinium applied to the stigma to produce the pollen-tube at full development. So that in the flower pollinated

the day of bloom fertilization takes place after 3-4 days. The seed ripens after about 14-15 days, while the dehiscence of the capsule occurs still later.

3. The ovule is rudimental at the time of bloom, but, differing from most orchids, the embryo-sac is completed without the stimulus of the pollentube.

4. The embryo-sac is four-nucleate, being derived from one megaspore.

5. The heterotype mitosis does not follow precisely either the parasyntaptic or the telosyntaptic type. The bivalent chromosome is formed when the univalent chromosomes come upon the equatorial plate.

6. A certain number of embryo-sacs is formed without the chromosome reduction.

7. The occurrence of the diploid egg is most probable, but in no case does parthenogenetic development take place.

8. The sterility of a certain number of ovules may be explained as being due to the omission of the chromosome reduction in forming the embryo-sac.

9. Double fertilization takes place. The endosperm nucleus is the product of the triple fusion of the male, synergid, and pole nuclei. It remains undivided. Fertilization is effected with the pollinium introduced into the ovarian cavity.

10. Both autonomic and aitionomic parthenocarpy may occur. The size of the resulting fruit depends on the nutritive condition, but under the same condition the aitionomic parthenocarpy gives rise to a larger fruit than the autonomic.

11. Parthenocarpy accompanies the development of the embryoless seed of normal structure.

12. On one and the same stock the aitionomic parthenocarpic fruit dehisces later than the autonomic, and in turn the latter dehisces later than the normal fruit. However, the latest dehiscence takes place on the autonomic parthenocarpic fruit developed on the unpollinated stock bearing a reduced number of flowers.

13. The seed-coat and the embryo can be brought to full development more or less independent of each other and of the fruit-wall.

14. Its own or foreign pollen-tubes do not promote the ovarial development alone, that is, without inducing the promoted development on more or less numbers of the ovule.

15. In the abscised flower the fertilized ovules can develop into normal seeds, while the ovary remains in all essentials as at the fertilization stage. This may afford an example of the acarpous seed formation.

16. At delayed fertilization of the ovule the synergid tends to develop into an adventitious embryo. In this case fusion between the male and synergid nuclei is highly probable.

17. Under a special condition the haploid egg may undergo the nuclear division leading to the generative parthenogenesis, but no cell division is ascertained.

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EXPLANATION OF PLATES.

PLATE V.

All the figures were magnified 1800 times.

Figs. 1-28. Archiesporium nuclei.

Fig. 1. Nuclear reticulum at the presynaptic stage (from a flower-bud 2 days before bloom).

Fig. 2. Beginning of contraction of the reticulum (same bud).

Fig. 3. Contraction advanced further (same bud).

Fig. 4. The midsynaptic stage; the synaptic knot appears somewhat homogeneous (same bud).

Fig. 5. Loosening of the spireme thread from the synaptic knot (a bud a day before bloom).

Fig. 6. Later stage (same bud).

Fig. 7. Surface view of a nucleus, showing a uniform peripheral distribution of the post-synaptic spireme (same bud).

Fig. 8. Network of the spireme in peripheral arrangement (from a flower a day after bloom).

Fig. 9. Condensation of the thread material into a certain number of groups, (a day after bloom).

Fig. 10. Surface view of a nucleus, showing the peripheral distribution of parallel threads (a day after bloom).

Fig. 11. Surface view of a nucleus, showing a breaking down of the network and a condensation of the thread material into groups (a day after bloom).

Fig. 12. Condensation of the thread material advanced further. A network structure is yet retained (a day after bloom).

Fig. 13. A more advanced state of condensation; groups of the thread material become more definite in appearance (a day after bloom).

Fig. 14. Formation of chromosomes from the condensed thread material. In this section of the nucleus 8 chromosomes with irregular outline and in a vacuolate condition are shown; the other 8 appear in the next section (2 days after bloom).

Fig. 15. A peripheral section of a nucleus, showing 6 alveolate chromosomes in peripheral arrangement; some of the chromosomes are rod-shaped (from a flower on the day of bloom).

Fig. 16. Two consecutive sections of a nucleus, showing more compact chromosomes, 16 in number (a day after bloom).

Fig. 17. Two consecutive sections of a nucleus, showing 16 chromosomes in a ring or loop form (a day after bloom).

Fig. 18. The same; some chromosomes are represented as parallel rods (a day after bloom).

Fig. 19. Two consecutive sections of a nucleus, showing 16 chromosomes becoming more compact (a day after bloom).

Fig. 20. A nucleus at the same stage; in each chromosome two spots take the colouring matter more heavily (a day after bloom).

Fig. 21. Two consecutive sections of a nucleus, showing 16 somatic chromosomes which appear bivalent; nucleolus disappears and the nuclear membrane becomes faint (same flower).

Fig. 22. Two consecutive sections of a nucleus, showing 16 homogeneous chromatin masses as chromosomes; a stage similar to Fig. 16 (a day after bloom).

Fig. 23. Two consecutive sections of a nucleus, showing compact but somewhat irregular chromosomes; nucleolus and the nuclear membrane disappear (a day after bloom).

Fig. 24. A nucleus at prophase, with chromosomes (18?) in the peripheral arrangement (a day after bloom).

Fig. 25. The same with chromosomes (16) retreating from the periphery (2 days after bloom).

Fig. 26. Transverse section of an archesporium with the chromosomes going to fuse pairwise at the equatorial plate (a day after bloom).

Fig. 27. Similar stage of an archesporium nucleus; 4 bivalent and 8 univalent chromosomes are shown (2 days after bloom).

Fig. 28. Surface view of an equatorial plate with 8 bivalent chromosomes (day of bloom).

Figs. 29-35. Somatic nuclei in the ovule.

Fig. 29. Spireme stage.

Fig. 30. Differentiation of the chromosome segments from the spireme.

Fig. 31. Formation of 18 univalent chromosomes.

Fig. 32. An equatorial plate with 16(?) chromosomes.

Fig. 33. Metaphase with 16 chromosomes.

Fig. 34. Daughter chromosomes at both poles of the spindle in polar view.

Fig. 35. The spindle at anaphase in side view.

PLATE VI.

Except Fig. 44, which was magnified 2250 times, all the figures were magnified 1500 times.

Figs. 36-54. First division in the archesporium.

Fig. 36. An equatorial plate with 8 bivalent chromosomes (day of bloom).

Fig. 37. Prophase of the homeotypic mitosis (?), showing a multipolar spindle with 16 univalent chromosomes (day of bloom).

Figs. 38-40. Metaphase showing 8 bivalent chromosomes (all from the same flower on the day of bloom).

Fig. 41. Early anaphase of the homeotypic mitosis, showing division in some of 16 univalent chromosomes (a day after bloom).

42. A spindle in side view, showing univalent chromosomes arranged in a ring (same flower).

Fig. 43. Early anaphase of the homoecotypic mitosis, showing some univalent chromosomes in division (a day after bloom).

Fig. 44. 16 univalent chromosomes at the same stage; two large problematic masses are shown (an unpollinated flower 4 days after bloom).

Fig. 45. Anaphase with 8 large chromosomes, of which one is yet undivided, and a small chromosome (?) undivided (day of bloom).

Fig. 46. Anaphase with two groups of 8 chromosomes further advanced (same flower).

Fig. 47. Anaphase of the homoecotypic mitosis, showing 16 distinct pairs of daughter chromosomes; a large problematic mass is found in one section (day of bloom).

Fig. 48. The same (a day after bloom).

Fig. 49. Polar view of 12 split chromosomes at one pole in the late anaphase (1 chromosome are found in another section) (a day after bloom).

Fig. 50. Two consecutive transverse sections of a spindle at the late anaphase, showing 16 daughter chromosomes at each pole in polar view (a day after bloom).

Fig. 51. Late anaphase with chromosomes in anastomosis; the chromosome group in the lower pole becomes larger in size than the upper (day of bloom).

Fig. 52. Telophase in oblique view, showing 16 chromosomes in each pole in interkinesis; in the lower pole 6 chromosomes are shown, the other 10 appearing in the next section (a day after bloom).

Fig. 53. A later stage (2 days after bloom).

Fig. 54. Formation of daughter cells (a day after bloom).

Figs. 55-60. Second division in the archesporium.

Fig. 55. Two consecutive sections of the lower daughter nucleus at prophase, showing the condensation of the chromatin material (2 days after bloom).

Fig. 56. Later stage, showing the formation of chromosome with the disappearance of the nuclear membrane and nucleolus (a day after bloom).

Fig. 57. Similar stage, showing 16 chromosomes in an irregular form (a flower 2 days after bloom).

Fig. 58. Prophase with compact 16 chromosomes (3 days after bloom).

Fig. 59. Equatorial plate in polar view, showing 8 chromosomes (day of bloom).

Fig. 60. Metaphase in side view; one of 8 chromosomes shows a split (3 days after bloom).

PLATE VII.

All the figures were magnified 1500 times.

Figs. 61-69. Second division in the archesporium.

Fig. 61. Metaphase with 8 split chromosomes (4 days after bloom).

Fig. 62. Early anaphase of the 16-chromosomic nucleus; in this section 12 pairs of split chromosomes are given (2 days after bloom).

Fig. 63. Anaphase, showing the total 16 pairs of daughter chromosomes (a day after bloom).

Fig. 64. 8 chromosomes at the pole of anaphase, showing their longitudinal fission (a day after bloom).

Fig. 65. Two consecutive sections of the spindle at anaphase, having 16 chromosomes at each pole (a day after bloom).

Fig. 66. Polar view of both upper and lower poles at anaphase, showing 16 chromosomes at each pole (2 days after bloom).

Figs. 67, 68. Anaphases with perhaps 8 chromosomes at each pole (a day after bloom).

Fig. 69. Formation of two daughter nuclei (day of bloom).

Fig. 70. A megaspore becoming an embryo-sac cell, with the nucleus at the resting reticular condition (3 days after bloom).

Fig. 71. A megaspore nucleus with the spireme converted into 16 chromosomes (day of bloom).

Fig. 72. Two consecutive sections of a megaspore nucleus at the multipolar stage, some of 16-18 chromosomes being represented in section (flower remained 4 days unpollinated).

Fig. 73. Metaphase of the same with 16 chromosomes in division; here only 13 pairs are represented (2 days after bloom).

Fig. 74. Anaphase of the same with 8 chromosomes (same flower).

Fig. 75. Late anaphase of the same (2 days after bloom).

Fig. 76. Telophase (a day after bloom).

Fig. 77. Formation of the daughter nuclei (2 days after bloom).

Fig. 78. Two daughter nuclei at resting condition (2 days after bloom).

Fig. 79. Metaphase of both daughter nuclei, showing 8 chromosomes at the equatorial plate (an unpollinated flower 4 days after bloom).

Fig. 80. The same stage, both spindles in side view (5 days after bloom).

Fig. 81. Early anaphase, the upper spindle in side view and the lower in polar view; both spindles possess 8 chromosomes, some of which are still undivided (flower remained 5 days unpollinated).

PLATE VIII.

All the figures were magnified 1500 times.

Fig. 82. Polar view of the equatorial plate of the lower and upper nuclei of the embryo-sac, 8 chromosomes in each nucleus are in division (2 days after bloom).

Fig. 83. One of the sections of a two nucleate embryo-sac, showing 8 chromosomes in polar view in the upper nucleus at anaphase and a peripheral longitudinal section of the lower spindle (same flower).

Fig. 84. Two nuclei at anaphase, the direction of their division being at right angle to each other (5 days after bloom).

Fig. 85. Formation of four nuclei in the embryo-sac; the lower pair are smaller (an unpollinated flower 5 days after bloom).

Fig. 86. Upper larger and lower smaller nuclei of the embryo-sac, being at the resting condition (2 days after bloom).

Fig. 87. Upper larger and lower smaller spindles of the embryo-sac, the upper in polar

view and the lower in side view. 14 chromosomes are visible in the upper spindle, showing the split of 8 chromosomes(?) (an unpollinated flower 3 days after bloom).

Fig. 88. A little later stage of the embryo-sac, both nuclei being at anaphase, the chromosomes of the lower spindle are in dissolution (an unpollinated flower 4 days after bloom).

Fig. 89. An embryo-sac at a still later stage, upper nucleus at late anaphase, and the lower being divided into two obliterated daughter nuclei (4 days after bloom).

Fig. 90. 4-nucleate embryo-sac; the lower two nuclei are in fusion (an unpollinated flower 5 days after bloom).

Fig. 91. The lower two nuclei from the 4-nucleate embryo-sac, showing successive stages of fusion.

Fig. 92. A lower spindle from a two-nucleate embryo-sac, showing the obliteration of the daughter chromosomes and the spindle fibres (3 days after bloom).

Fig. 93. An embryo-sac showing the formation of an egg apparatus (5 days after bloom).

Fig. 94. Same stage (unpollinated flower 5 days after bloom).

Fig. 95. Differentiation of an egg nucleus and synergid nuclei (5 days after bloom).

Fig. 96. Apical view of a complete embryo-sac, showing the egg apparatus in polar view (3 days after bloom).

Fig. 97. A complete embryo-sac (unpollinated flower 5 days after bloom).

Fig. 98. Entry of a pollen-tube into the embryo-sac, with the tube nucleus and a male nucleus in its apical portion (5 days after bloom).

Fig. 99. Fertilized embryo-sac; a male nucleus having entered the egg is visible beneath the female nucleus (4 days after bloom).

PLATE IX.

All the figures, except Figs. 105, 108, 109, and 114, were magnified 1500 times.

Fig. 100. Two consecutive sections of a fertilized embryo-sac (a flower 4 days after bloom).

Fig. 101. Fertilized embryo-sac; fusion takes place between the male and female nuclei, but the male, a synergid, and the polar nuclei remain yet unfused (4 days after bloom).

Fig. 102. A little later stage in polar view (a flower pollinated 4 days after bloom and taken 5 days after pollination).

Fig. 103. The first metaphase in the oosphere, with 16 chromosomes in division (a similar flower).

Fig. 104. Two-celled proembryo; the upper nucleus in metaphase with 16 chromosomes on the equatorial plate (6 days after bloom).

Fig. 105. Embryo-sac with the two-celled proembryo and two nuclei for fusion (same flower as in Fig. 103). $\times 750$.

Fig. 106. Polar view of the early anaphase of the first mitosis of the oosphere, showing 16 chromosomes splitting into 32 daughter halves (same stage as Fig. 103) (same flower as in Fig. 103).

Fig. 107. An epidermal cell of the multinucleate embryo, showing 16 distinct chromosomes on the equatorial plate (10 days after bloom).

Figs. 108, 109. Embryo-sacs with two embryos, one of which derived from a synergid (flowers pollinated 4 days after bloom). $\times 750$.

Figs. 110-113. Nuclear division in the unfertilized haploid eggs.

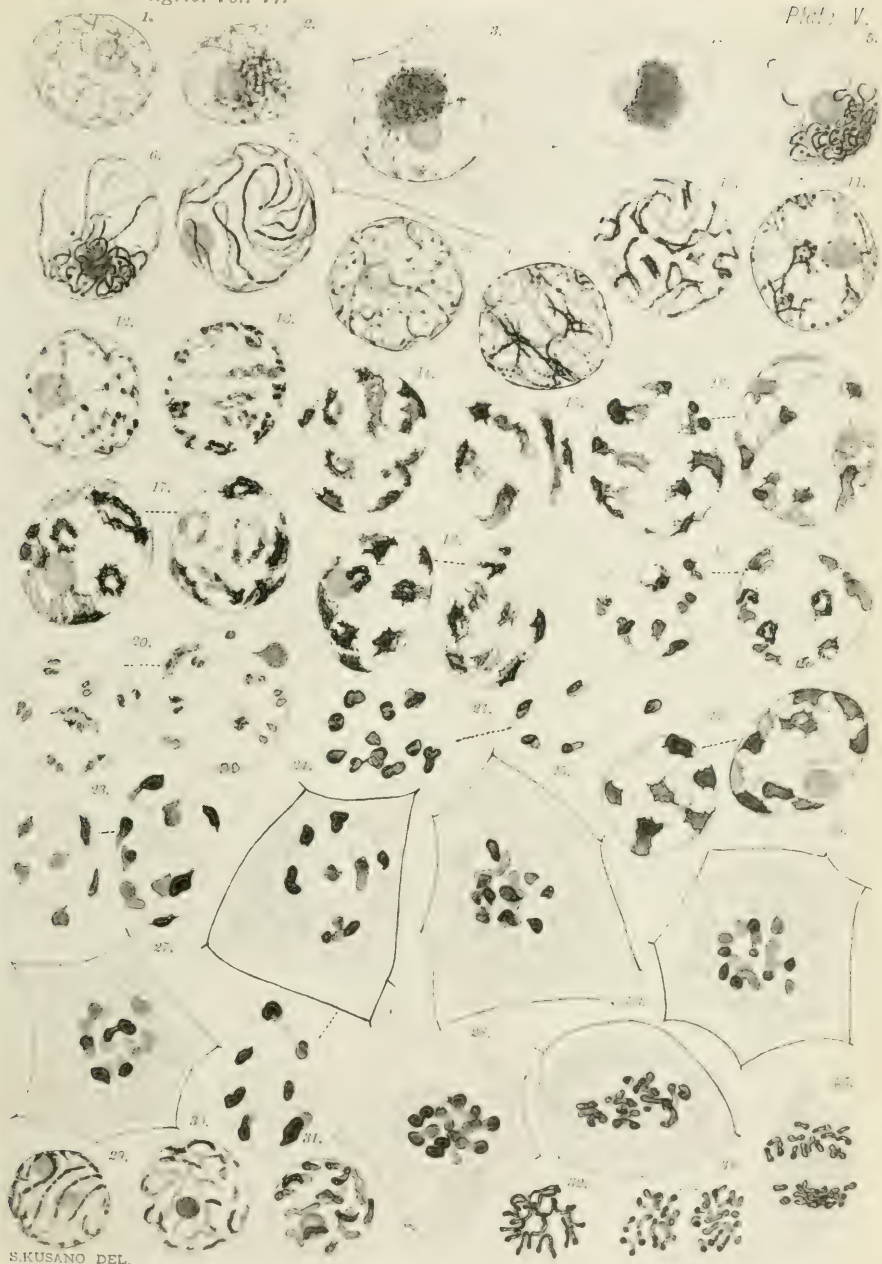
Fig. 110. Metaphase showing 8 chromosomes (6 days after pollination with the *Bletia*-pollinium).

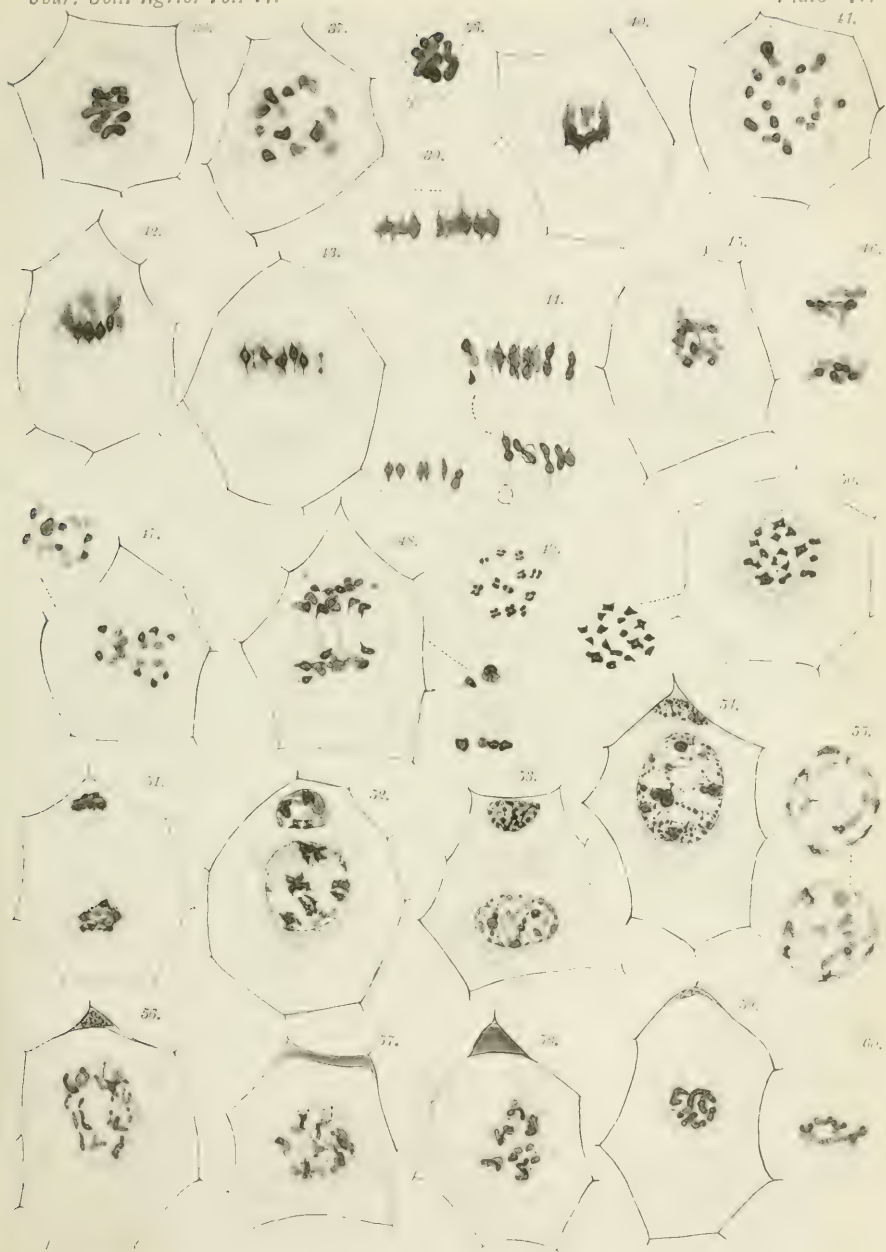
Fig. 111. Metaphase showing the same number of chromosomes in longitudinal fission (a flower from the unpollinated stock, 12 days after bloom).

Fig. 112. Anaphase (flower under similar condition as in Fig. 110).

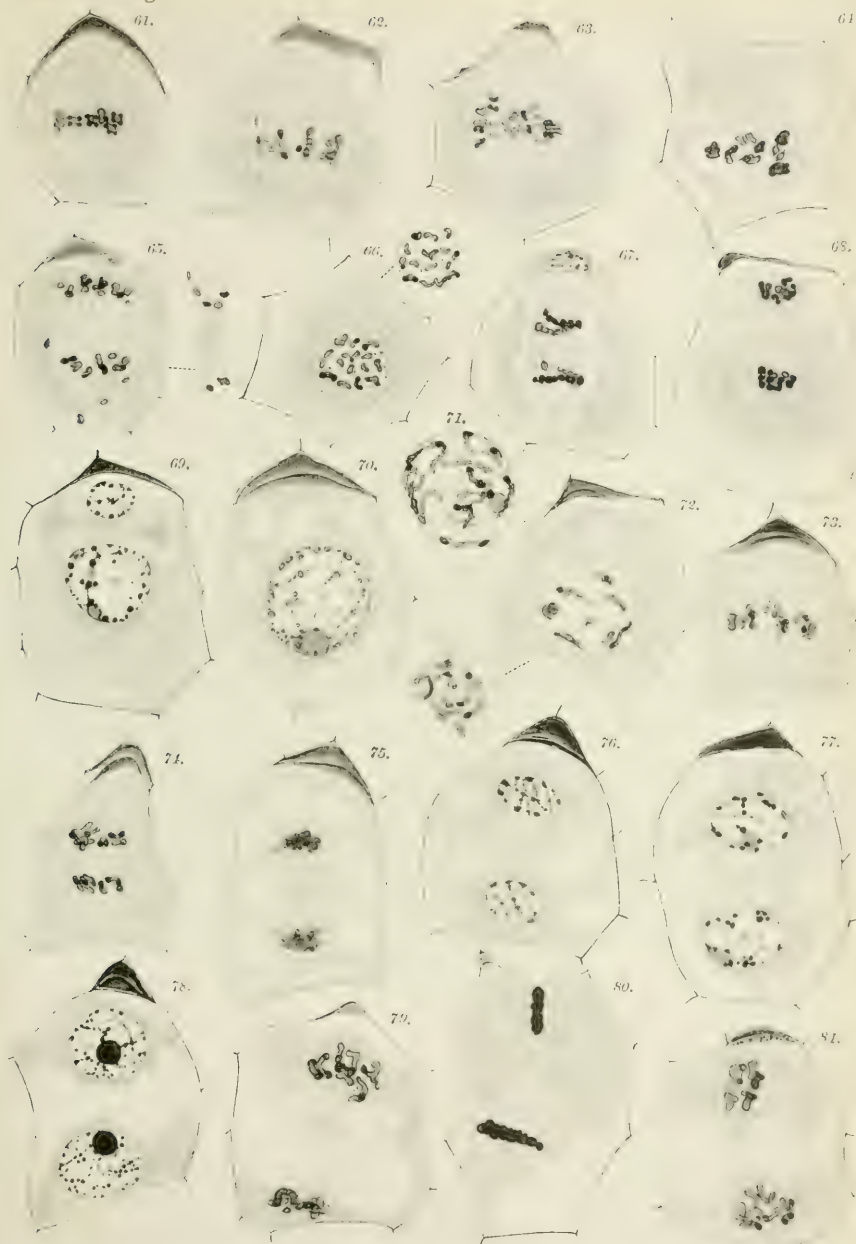
Fig. 113. Later anaphase (flower under similar condition as in Fig. 110).

Fig. 114. A young embryo and the ovular tissue surrounding the embryo-sac. \times ca. 500.













On the Influence of Nutrition upon the Development of Sexual Organs in the Fern Prothallia.

BY

Isaburo Nagai.

With Plate X and seven Text-Figures.

The writer (1913) has shown that the formative factors of environment play important roles in the sex development in the prothallia of *Ceratopteris thalictroides* and of other ferns. It is attempted in the present paper to show quantitatively the influence of nutrition on the development of the sexual organs in the gametophytes of *Osmunda regalis* var. *japonica* Milde and of *Asplenium Nidus* L.

I. The Influence of the Concentration of Knop's Solution.

A. ASPLENIUM NIDUS L.

The spores used for the experiments were collected in April, 1914 from a single potted specimen in the conservatory of the Horticultural Department in the College of Agriculture, and they were sown immediately in the Petri dish which contained 0.35 per cent of KNOP's solution. The culture was kept near the west window of the laboratory giving only a diffused light. Nineteen days later, the germinated spores developed to the young prothallia of two to three cells. The culture was kept unchanged till July 2nd and up to this time, the prothallia were found to be sterile, though the growth was perfectly normal. They were atheristic, bearing neither archegonia nor antheridia.

These prothallia were transplanted to the different cultures which contained 30 c.c. of 0.7 %, 0.35 %, 0.175 %, 0.058 %, 0.017 % of freshly prepared KNOR's solution* and distilled water respectively. The cultures were kept near the north windows of the institute, thus they were exposed only to a diffused light. Care was taken to maintain the uniformity of light and other external conditions which might influence the growth of prothallia besides the concentration of the nutrient solution. The contamination of some green algæ was unavoidable, but their growth never reached to such an extent as to give any serious change in the growth condition of the prothallia. From the end of July to the middle of August, the room temperature had been extremely high and sometimes reached over 32°C. The conditions at twenty four days after transplanting are summarised as follows.

Cult. No.	Conc.	Condition
I	0.7 %	Vigorous growth, meristem differentiated, many developed to the normal, heart-shaped, meristic prothallia. Very few antheridia initials. No archegonia.
II	0.35 %	Vigorous growth, same as I. Many antheridia, but no archegonia.
III	0.175 %	Many antheridia and few archegonia.
IV	0.058 %	Many prothallia, meristic but sterile.
V	0.0175 %	Some prothallia meristic but majority remains ameristic and sterile.
VI	distilled water	Ameristic and sterile, pale yellowish green color.

On August 8th the first measurement of the number of antheridia and archegonia was made. Individuals were taken at random from the different parts in the culture, avoiding the unconscious selection of any particular group of individuals. The result is given in the Table I. It must be noted that besides the number of individuals recorded in the table, a number of more prothallia were examined under microscope so as to insure the sterility of the population. For example, only 55 individuals are recorded to be sterile under VI, but in reality more than a hundred other individuals were examined and were also proved to be sterile.

*The formula is given on Sec. II.

Table I. *Asplenium Nidus*.

The number of archegonia and antheridia (observed Aug. 6, spores sown April 23, transplanted July 2).

Cult. No.	Conc.	Total proth.	Total anth.	Average per prothallium	Total archeg.	Average per prothallium
I	0.7	95	1629	17.14	38	0.40
II	0.35	71	931	13.11	58	0.81
III	0.175	89	1493	16.77	72	0.808
IV	0.058	100	620	6.20	2	0.02
V	0.0175	61	6	0.09	0	0
VI	dis. water	55	0	0	0	0
Total		471	4679	9.93	170	0.36

Table II. *Asplenium Nidus*.

The number of sterile and fertile prothallia.

Cult. No.	Conc.	Total proth.	Actual number of proth.			No. of proth. in per cent.		
			monoecious and female	male	sterile	monoecious and female	male	sterile
I	0.7	95	8	79	8	8.4	83.2	8.4
II	0.35	71	11	53	7	15.5	74.6	9.9
III	0.175	89	10	73	6	11.2	82.1	6.7
IV	0.058	100	1	67	32	1.0	67.0	32.0
V	0.0175	61	0	1	60	0	1.6	98.4
VI	dis. water	55	0	0	55	0	0	100.0
Total		471	30	273	168	6.35	57.96	35.67

The alternative variations are calculated in the following alternatives from the same material given in the preceding tables.

1. Monoecious (both archegonia and antheridia bearing) + female (only archegonia bearing) prothallia vs. male (only antheridia bearing) + sterile prothallia.

2. Male vs. all others (monoecious, female, and sterile prothallia).

3. Sterile vs. fertile prothallia (compare Text-fig. 1).

4. Total number of archegonia vs. total number of antheridia.

Table III. *Asplenium Nidus*.

Alternative variations (1, 2, 3).

Cult. No.	Conc.	1			2			3		
		(monocious+female)	(male+sterile)	δ^*	(male)	(monocious+sterile)	δ^*	(sterile)	(fertile)	δ^*
		0	1		0	1		0	1	
I	0.7	8.1	91.6	27.74	83.2	16.8	37.39	8.4	91.6	27.74
II	0.35	15.5	84.5	36.19	74.6	25.4	43.53	9.9	90.1	29.87
III	0.175	11.2	88.8	31.54	82.1	17.9	38.33	6.7	93.3	25.00
IV	0.058	1.0	99.0	9.95	67.0	33.0	47.02	32.0	68.0	46.65
V	0.0175	0	100	0	1.6	99.4	12.61	98.4	1.6	12.55
VI	dis. water	0	100	0	0	100	0	100	0	0

Table IV. *Asplenium Nidus*.

Alternative variation (4).

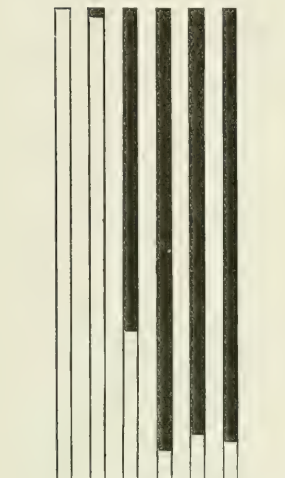
Cult. No.	Conc.	Actual number of		Total	(Archeg.)	(Anth.)	δ
		Archegonia	Antheridia		0	1	
I	0.7	38	1629	1667	2.3	97.7	14.93
II	0.35	58	931	989	5.9	94.1	23.56
III	0.175	72	1493	1565	4.6	95.4	20.95
IV	0.058	2	620	622	0.3	99.7	5.47
V	0.0175	0	6	6	0	100	0
VI	dis. water	0	0	0	0	0	0

As we see in the preceding tables, the most favorable concentration for the formation of antheridia and archegonia lies somewhere between 0.35 per cent to 0.175 per cent. There are found 16 antheridia and 0.8 archegonia per prothallium in that concentration, whereas in 0.7 per cent, 17.14 antheridia and 0.4 archegonia, and in 0.35 per cent 13.11 antheridia with 0.81

* δ =standard deviation= $\sqrt{p_1'0\% \times p_2'0\%}$. For particulars see JOHANNSEN: Elemente der Exakten Erblchkeitslehre, 1913, p. 61 et seq.

archegonia are found. The size of prothallia is also largest in those which are grown in the 0.175 per cent solution. It might be that the comparative

W V IV III II I



Text-fig. 1. Diagram showing in per cent sterile and fertile prothallia grown in the different concentrations of KNO₃ solution. Black parts in the columns indicate the percentage of fertile and white parts sterile prothallia.

low number in the antheridia and archegonia found in 0.35 per cent culture is due to the unguarded error in the selection of the population for counting. A comparatively younger individuals might have been taken, for in all cultures, it has been observed that the stages in growth in the individual prothallia are somewhat diverse at the different parts in the culture.

Thus it is clearly shown that the development of sexual organs is dependent upon the concentration of the culture media. This evidence can further be confirmed by the fact that in the higher concentrations (0.35 per cent up) many abortive ameristic prothallia which are found in a mass, are strongly "male" and bear abundant antheridia. Whereas those prothallia in the lower concentration (0.0175 per cent) the similar abortive prothallia bear very few antheridia or in many cases are purely sterile.

The distributions of the antheridia and archegonia in the populations of the different cultures are also variable (see Table V).

Table V. *Asplenium Nidus*.*

Frequency of distribution of antheridia (Aug. 6-7).

Cult. No.	Conc.	2 (0-4)	7 (5-9)	12 (10-14)	17 (15-19)	22 (20-21)	27 (25-29)	32 (30-31)	37 (35-39)	42 (41-44)	47 (45-49)	
I	0.7	13	8	19	20	13	8	6	5	2	1	95
II	0.35	16	12	12	13	10	3	3	1	1	0	71
III	0.175	13	10	16	17	13	7	4	4	3	2	89
IV	0.058	45	25	19	5	3	2	1				100
V	0.0175	60	1									61
VI	dis. water	55										55
Total		202	56	66	55	39	20	14	10	6	3	471

In the higher concentrations the modal classes lie in the class 17 except for the Culture II, but the mode shifts to the lowest class as the concentration of the nutrient solution decreases and in the population of the last three cultures a marked increase in the number falling in the lowest class (2) is noted and especially for the last two, the entire population comes under the lowest class except in the case with Culture V, where only one individual is found to bear eight antheridia and the rest is nothing but all sterile individuals. The reader will appreciate the matter more fully in comparing the accompanying graphic representation in Text-figure 2. The following biometric constants show the general trend of variability in the distribution of the antheridia in the different cultures.

Table VI.† *Asplenium Nidus*.

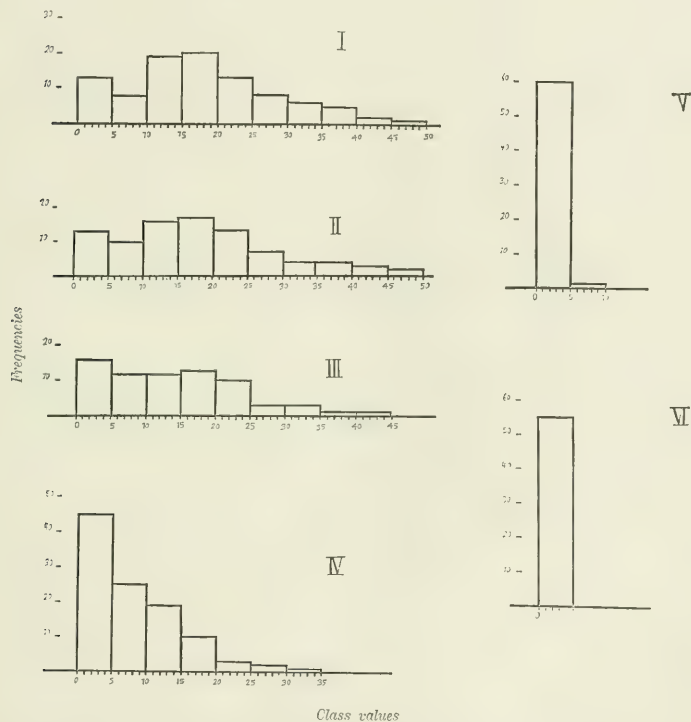
The variation in the number of antheridia.

Cult. No.	Conc.	M	s	C
I	0.7	17.474 ± 0.7379	10.664 ± 0.5218	61.019 ± 3.9432
II	0.35	13.479 ± 0.7641	9.546 ± 0.5422	70.818 ± 6.9478

Each class value indicates the number of antheridia per prothallium and the figures under each class indicate the number of prothallia which bear corresponding number of antheridia.

† M =mean, s =standard deviation, C =coefficient of variability.

Cult. No.	Conc.	M	δ	σ
III	0.175	17.168 ± 0.8521	11.783 ± 0.5956	68.633 ± 6.0353
IV	0.058	7.30 ± 0.4366	6.474 ± 0.3087	88.682 ± 6.7843
V	0.0175	—	—	—
VI	dis. water	—	—	—
Total population		10.896 ± 0.3656	10.429 ± 0.2585	95.711 ± 3.5103



Text-fig. 2. *Asplenium Nidus*. The histogram showing the frequency of the distribution of antheridia in the populations grown in the different concentrations of Knop's solution. I (0.7%), II (0.35%), III (0.175%), IV (0.058%), V (0.0175%), VI (dis. water).

It is observed that there exists a dioecious appearance among the fully grown prothallia. The "female" prothallia are always large and meristic. They bear antheridia but the number is usually very small and in many cases they are entirely free from antheridia, thus being purely dioecious "female." Besides, some large, though sterile, meristic prothallia are often found. They are easily distinguishable because of their good development of the thallus and the absence of sexual organs. Evidently they are the younger stage of "female" prothallia which in later stages form archegonia with or without accompaniment of antheridia. Thus the archegonia-bearing prothallia seem to be differentiated from the early stage of their development. It seems that there exists a mutual relation between the archegonia and the antheridia forming capacity in the prothallia. When the antheridia are formed abundantly, the archegonia is suppressed, whereas when the archegonia are formed, the development of the other sex organ is entirely or partially suppressed. In this way there must have a negative correlation between the formation of archegonia and antheridia. A fuller discussion on this point will be given later. Further, the fact that the "female" and monoecious prothallia (only archegonia bearing and both antheridia and archegonia bearing) are always meristic suggests the existence of the following relation,—that the differentiation of the cells of the prothallia into meristic and ameristic i. e. differentiation of growing point, is in some way correlated with the formation of the archegonia. Some unknown physiological conditions in the cell which bring the differentiation of the growing point in the thallus eventually give rise to the inner physiological changes by which the archegonia are developed. We do not know the nature of the factors concerned in the differentiation of the prothallial cells, but it seems clear that the formation of meristem is only initiated where the nutrition is good. If the growth condition is unfavorable to the normal development of prothallia, either by the excess or the scarcity of the nutrition, the differentiation of meristem never takes place. Under such conditions the prothallia are remained to be ameristic, mostly irregular in shape, and bear only antheridia or sometimes the entire suppression of their development occurs. Humidity, temperature, light intensity and transpiration also play the important role in it. It is safe to assume therefore, that the favorable nutrition acts, at

least in indirect ways, as the determining factor for the archegonia formation, for as we have seen already, the meristem differentiation is only possible under favorable growth conditions and the meristem formation ultimately brings about the development of the archegonia on the same prothallia.

The distribution of the archegonia in the different populations at the different stages of the development is given in the following tables.

Table VII. *Asplenium Nidus*.*

Frequency in the distribution of archegonia (Aug. 6th-7th).

Cult. No.	Conc.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
I	0.7	1	1	1	0	3	0	1	0	0	1					8
II	0.35	1	1	2	2	1	0	0	1	2	1					11
III	0.175	1	1	0	1	1	0	1	1	1	1	0	1	0	1	10
IV	0.058		1													1
V	0.0175															0
VI	dis. water															0
Total		3	4	3	3	5	0	2	2	3	3	0	1	0	1	30

Table VIII. *Asplenium Nidus*.*

Ditto. The condition in a month later (Sept. 4th-8th).

Cult. No.	Conc.	3 (1-5)	8 (6-10)	13 (11-15)	18 (16-20)	23 (21-25)	28 (26-30)	33 (31-35)	38 (36-40)	43 (41-45)	48 (46-50)	
I	0.7	14	7	3	1	1	1	1				28
II	0.35	11	9	4	3	1	0	1				29
III	0.175	24	11	4	6	4	3	1	1	1	1	57
IV	0.058											0
V	0.0175											0
VI	dis. water											0
Total		49	27	11	10	6	4	3	1	1	1	114

* See explanation of Table V.

The marked increase in the number of archegonia formed by the individual prothallium as well as the total number for a population in the more matured stage is noted. In the first measurement, the highest class in the number of archegonia produced by a single prothallium was only 14, whereas in the last measurement the value increased as high as 53. The average number per prothallium also increased more than three times (compare Table I with Table IX). In comparing with the development of antheridia, it is shown to be that the development of archegonia requires much more time.

Table IX. *Asplenium Nilus*.

The formation of archegonia (Sept. 4th-8th).

Cult. No.	Conc.	Total proth.	Arch. bearing prothallia	male and sterile proth.	Total archegonia	Average per prothallium
I	0.5	123	28	95	189	1.53
II	0.35	135	29	106	262	1.94
III	0.175	249	57	192	898	3.60
IV	0.058	111	0	111	0	0
V	0.0175	102	0	102	0	0
VI	dis. water	150	0	150	0	0
Total		870	114	756	1349	1.5

Reference has already been made to the existence of dioecious tendency and the negative correlation between the archegonia and antheridia formation. As the following figures in Table X show clearly, about half of the individuals having archegonia are strictly dioecious, i. e. at the same time having no antheridia. Out of 114 archegonia-bearing prothallia, 54 bear only archegonia but no antheridia; the remainder bears the archegonia as well as the antheridia i. e. monoecious. Further it is observed that this is due to a peculiar phenomena of changing sex appearance according to the age of prothallia. Many archegonia-bearing ("female") prothallia which are classed under 0 class in respect to the antheridia in Table X, are in reality monoecious. At the basal part of the prothallia, rudiments of the dead, brownly colored antheridia are often found, but the exact number of them are

impossible to count because the thallus cells are also brownly colored in this region. Or in many cases, the formally antheridia-bearing region at the basal part has apparently been detached, perhaps by decaying; and only archegonia bearing prothallia are in reality a part of restituted old "male" prothallia. Thus the sexual character of the prothallia is changed at the different stages of their development. In the younger stage, they are "male" and in the later stage they become "female"; in another words, they may be said to be successively dioecious. Besides, many individuals are found to have both sexual organs at the same time. All these differences seem due to the influences of external factors. The correlation between the archegonia and the antheridia is negative as much as -0.28719 expressed by the coefficient of correlation.

Table X. *Asplenium Nidus*.

Correlation between the number of archegonia and antheridia.

Culture I (0.7 %).—Sept. 4th.

$\begin{matrix} X(\delta) \\ Y(\varphi) \end{matrix}$	2	7	12	17	22	27	32	37	42	47	52	57	62	
2	4		1	1	2	2			1	1			1	13
7	1		1	1	2				1					6
12	2	1												3
17	3													3
22	1													1
27	1													1
	12	1	2	2	4	2	0	0	2	1	0	0	1	27

Culture II (0.35 %).—Sept. 4th.

$\begin{matrix} X \\ Y \end{matrix}$	2	7	12	17	22	27	32	37	42	47	52	57	62	
2	7							1	2					10
7	4		1		2	1		1						9
12	3						1	1						5
17	2	1												3
22														0
27	1													1
32	1													1
	18	1	1	0	2	1	1	3	2	0	0	0	0	29

Culture III (0.175 %).

Y \ X	2	7	12	17	22	27	32	37	42	47	52	57	
2	6	1		2		1	3	3		4			20
7	5		1	1	1		1	1	1	1			12
12	3			1						1		1	6
17	2	1		1	1	1							6
22	1			1	1	1							4
27	2			2		1							5
32													0
37	1												1
42	2												2
47			1										1
52	1												1
	23	2	2	8	3	4	4	4	1	6	0	1	58

Total of Culture I, II, and III.

Y \ X	2	7	12	17	22	27	32	37	42	47	52	57	62
2	17	1	1	3	2	3	3	4	3	5			1
7	10		3	2	5	1	1	2	2	1			
12	8	1		1			1	1		1		1	
17	7	2		1	1	1							
22	2			1	1	1							
27	4			2		1							
32	1												
37	1												
42	2												
47			1										
52	1												
	53	4	5	10	9	7	5	7	5	7	0	1	1

X class=the number of the antheridia.

Y " = " " " " " archegonia.

$$M_x = 16.035 \pm 1.0298$$

$$M_y = 10.5087 \pm 0.6712$$

$$\hat{\sigma}_x = 16.287 \pm 0.7215$$

$$\hat{\sigma}_y = 10.4744 \pm 0.4716$$

$$r = -0.28719 \pm 0.08131$$

B. *OSMUNDA REGALIS* L. VAR. *JAPONICA* MILDE.

The spores were collected in the spring of 1914 from the different individuals growing wild in the University grounds. The prothallia which are used for the experiments were taken from the individuals grown originally in the modified KNOR's solution lacking in magnesium salt (see Sec. II.). Up to the time of transplanting, the prothallia had been grown normally but no sexual organs were present. The nutrient solutions and the other conditions were the same as those described in the case with *Asplenium*. The spores were sown on May 14th in Solution VI (see Sec. II.), and transplanted on July 2nd. The condition at thirty-five days after the transplantation is given in the following tables.

Table XI. *Osmunda regalis* var. *japonica*.

(Observed Aug. 6-7. Spores sown May 14, transplanted July 2).

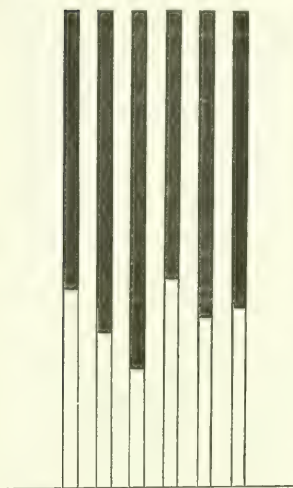
Cult. No.	Conc.	Total proth.	Total anth.	Total arch.	Anth. average per proth.	Archeg. av. per proth.
I	0.7	97	588	0	6.06	0
II	0.35	122	752	0	6.16	0
III	0.175	95	470	0	4.94	0
IV	0.058	76	266	0	3.50	0
V	0.0175	86	267	0	3.10	0
VI	dis. water	86	145	0	1.68	0
Total		562	2488	0	4.43	0

Table XII. *Osmunda regalis* var. *japonica*.

Alternative variation. Sterile vs. male prothallia.

Cult. No.	Conc.	Total proth.	Sterile proth.	Male proth.	(sterile)	(male)	δ
					0	1	
I	0.7	97	37	60	33.1	61.9	48.56
II	0.35	122	44	78	36.1	63.9	48.03
III	0.175	95	42	53	44.2	55.8	49.66
IV	0.085	76	19	57	25.0	75.0	43.30
V	0.0175	86	28	58	32.6	67.4	46.86
VI	dis. water	86	36	50	41.9	58.1	49.34

VI V IV III II I



Text-fig. 3. Diagram showing in percent sterile and male prothallia grown in Knor's solution of the different concentrations. Black parts in the columns indicate the percentage of male prothallia.

The archegonia have not developed up to this time. The development is much slower in *Osmunda* than in *Asplenium*; though the difference in the nutrient solution in the early stage of the development of the prothallia must be taken into consideration.

The development of antheridia comes under a similar category as in *Asplenium*. The size of the antheridia crop decreases as the concentration of the nutrient solution decreases. It must be noted, however, that in *Osmunda* the antheridia formation is quite possible even in the lower concentration whereas in *Asplenium* this is not the case. PRANTL (1881) has already shown that the spores of *Ceratopteris thalictroides* which are rich in reserve materials are able to form the meristic prothallia for a while, even if they are grown in the nutrient solution lacking in nitrogen, but the prothallia of *Osmunda regalis*, *Polypodium vulgare*, and *Aspidium*

Filix mas are unable to form meristem in the similar solution. The writer (1913) has also shown that the prothallia of *Ceratopteris thalictroides* are able to form the antheridia without addition of nutrient salts from without. Considering these evidences at hand, it seems certain that the reserve materials stored in the spores play an important role in the antheridia formation under insufficient nutrition. The spores of *Asplenium Nidus* are much smaller than those of *Osmunda* and evidently they must contain less amount of reserve materials which would supply some necessary substances for the antheridia formation.

The relation that exists between the meristem differentiation and the archegonia formation holds good in *Osmunda* as in *Asplenium Nidus*. The

poor nutrition which allows the prothallia to grow only ameristic, is the determining factor for the archegonia formation in so far as the other growth conditions are the same.

The high concentration as well as the low concentration is inhibitory for the development of archegonia and also reduces the number of antheridia formed. In another set of cultures, it was shown that the prothallia which have previously been cultivated in 0.6 per cent KNOP's solution (Culture C) and transplanted in 2 per cent, they produced a very small amount of antheridia. The cells of these prothallia became roundish, and the short rhizoids grew from the upper portion of the prothallia and which is usually not the case with those which are grown in lower concentration.

Cult. No.	Conc.	Total proth.	Sterile proth.	Antheridia	Average per proth.
C	0.6 %	105	36	165	1.57
I	2.0	75	71	7	0.09
II	0.5	81	7	632	7.80

The above measurement was made seventy five days after the transplantation was made. A marked increase in the average number of antheridia in Culture II seems to be due to the freshness of the nutrient solution which gave new start for growth.

The distribution of antheridia in the different populations is given in the Table XIV (compare Text-fig. 4).

Table XIV. *Osmunda regalis* var. *japonica*.

Frequency of distribution of antheridia.

Cult. No.	Conc.	2 (0-4)	7 (5-9)	12 (10-14)	17 (15-19)	22 (20-24)	27 (25-29)	32 (30-34)	37 (35-39)	
I	0.7	54	19	10	5	6	1	1	1	97
II	0.35	66	23	13	14	5	2			123
III	0.175	53	20	12	7	3				95
IV	0.058	50	21	5						76
V	0.0175	61	16	9						86
VI	dis. water.	74	11	1						86
Total		358	110	50	23	14	3	1	1	563

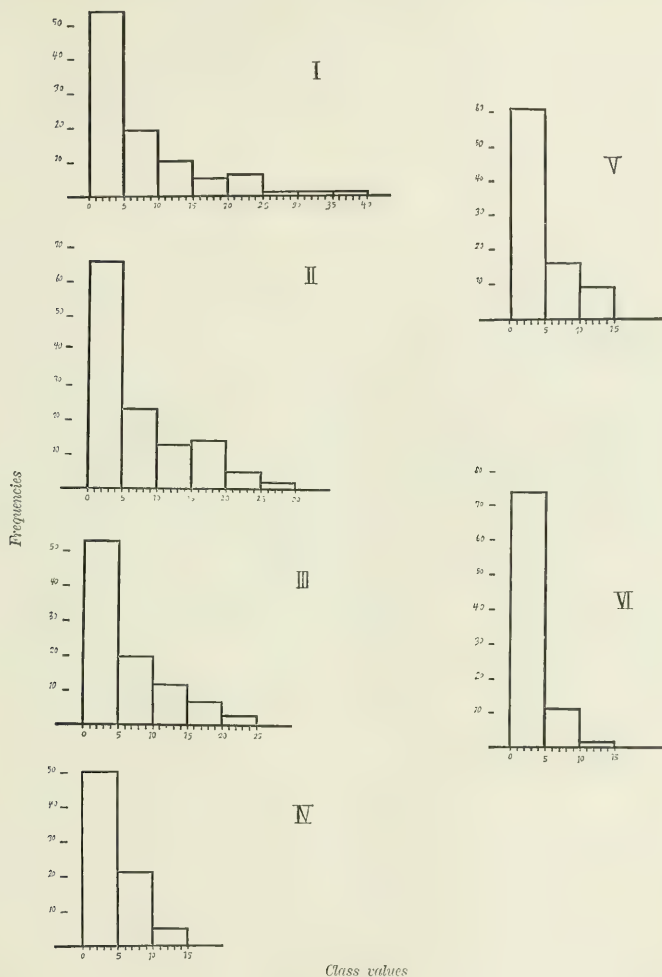
The biometrical constants are as follows :

Cult. No. I	Conc.	M	S	C
I	0.7	6.948 ± 0.5093	7.437 ± 0.3602	107.034 ± 9.4023
II	0.35	6.910 ± 0.3091	6.563 ± 0.2823	94.867 ± 6.1876
III	0.175	6.053 ± 0.3833	5.539 ± 0.2714	80.525 ± 5.9679
Total population		5.364 ± 0.1564	5.504 ± 0.1106	102.610 ± 3.6341

The coefficients of variabilities in *Osmunda regalis* are much greater than those of *Asplenium Nidus*. As we see in the above table, C for the population in Culture I and that for the total population of all six cultures exceed by more than 100 per cent, that is to say, the variability of the population is more than one hundred per cent of its own mean. This is rather unusual, though such a case is also found in the fertility in the fruit of *Crinum Longifolium* (HARRIS, 1912, p. 80).

The archegonia are found only in Culture II and III. In the higher concentrations, many meristic prothallia are found but the archegonia are not found in them. It seems that the possibility of archegonia formation is much less in the experimented material of *Osmunda* than that of *Asplenium*, though it is not certain whether this is also true in free nature.

The idea was suggested that the liquid culture might be unfavorable to the development of archegonia so that the culture with the solid media might give better result. Thus the sand culture was tried. The well developed prothallia grown in KNOP's solution and bore nothing but the antheridia were transplanted upon pure sand containing the same amount of KNOP's solution of the same concentration as in the liquid culture. A month later, the cultures were examined but the result was not very positive. Only four archegonia were found in the sand culture of 0.25 per cent KNOP's and the rest series of cultures consisting of 1.0 per cent, 0.5 per cent, 0.002 per cent and the clay soil were all found to be destitute of archegonia. Perhaps it may require a longer time to bring the prothallia to a fruitful condition. The condition in the liquid cultures was as follows :



Text-fig. 4. *Osmunda regalis* var. *japonica*. Histogram showing the frequency of the distribution of antheridia in the populations grown in Knop's solution of the different concentrations (compare Table XIV).

Table XV. *Osmunda regalis* var. *japonica*.

Cult. No.	Conc.	Archegonia	Remarks
I	0.7	—	Many meristic prothallia.
II	0.35	40
III	0.175	4
IV	0.058	—
V	0.0175	—	Few
VI	dis. water	—	Very few

It is observed that where the ameristic, filamentous prothallia which previously grown in the liquid culture are transferred to the sand culture, they are able to form one or more meristems from the different parts of prothallia. Each of these meristic parts are notched and normally heart shaped bearing abundant antheridia and few rhizoids (see Text-fig. 5). When they are fully developed, they are detached from the older portion at the slender parts, thus the prothallia of *Osmunda* are able to propagate themselves purely in an asexual manner.

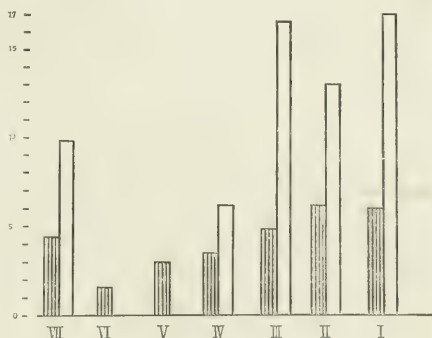


Text-fig. 5. *Osmunda regalis* var. *japonica*. A stage in the vegetative propagation of a prothallium.

In comparing the data thus far obtained in the two species, we see that the average number of antheridia produced per prothallium is much less in *Osmunda* than in *Asplenium* (compare Text-fig. 6). The antheridia producing capacity seems stronger in the latter, even if they are grown with the same strength of the nutrient solution under the similar other external conditions. There is a gradual decrease in the number of the sterile prothallia in *Asplenium Nidus* as the concentrations of the nutrient solutions decrease. The reaction of the nutrient strength on the sex development is thus directly manifested, but in the case with *Osmunda* the reaction is not so regular as in the case with the former. No corresponding increase in the number of sterile prothallia according to

the decrease in the concentration of the nutrient solution is in any way manifested which, however, is the case with *Asplenium Nidus*. This is perhaps due to the fact that the spores of *Osmunda* may contain more necessary reserve materials for the sex development than that of *Asplenium*, thus the external influences may not be so regularly manifested. As the antheridia formation is possible in the lower concentration in *Osmunda* which is lacking in *Asplenium*, the standard deviations of the alternative variations between the sterile and fertile prothallia in the lower concentrations are always higher in the populations of *Osmunda* than those of *Asplenium Nidus*. As the majority of population belongs to either one of the two classes, the value of δ approaches to zero.

The different tables given in the previous pages are shortly summarized here.



Text-fig. 6. Diagram showing the influence of concentration of Knor's solution upon the formation of antheridia in *Osmunda* (shaded column) and in *Asplenium* (white column). The height of column indicates the average number of antheridia per prothallium. The figures at the foot of each column indicate the culture number. I (0.7%), II (0.35%), III (0.175%), IV (0.085%) V (0.0175%), VI (dis. water), VII (Total).

Average antheridia per prothallium.	<i>Osmunda</i>	<i>Asplenium</i>
I	6.06 ¹	17.14 ²
II	6.16	13.11
III	4.94	16.77
IV	3.50	6.20
V	3.10	0.09
VI	1.68	0
Total	4.42	9.93

1. Table XI.

2. Table I.

Average archegonia per prothallium.

I	— ¹	0.40 ²
II	40 ³	0.81
III	4 ⁴	0.808
IV	—	0.02
V	—	0
VI	—	0
Total	—	0.36

Coef. of variability (C) in the number of antheridia.

I	107.031±9.4026 ⁵	61.019±3.9432 ⁶
II	91.863±6.1876	70.818±6.9478
III	80.525±5.9679	68.633±6.0353
IV	—	88.682±6.7813
V	—	—
VI	—	—
Total	102.610±3.6341	95.711±3.5403

Alternative variation (δ) sterile vs. fertile prothallium.

I	48.56 ⁷	27.74 ⁸
II	48.03	29.87
III	49.66	25.00
IV	43.30	46.65
V	46.86	12.55
VI	49.34	0

II. The Influence of the different Kinds of Nutrient Solutions.

In order to compare the influence of the different nutrient solutions, the spores of *Osmunda regalis* var. *japonica*, the same material used for the previous experiments, are sown in the following nutrient solutions:

1. Table XV.
2. Table I.
3. Total number.
4. Total number.
5. Table XIV.
6. Table V.
7. Table XII. sterile vs. male.
8. Table III.

I. The full nutrient solution (KNOP'S). II. Nitrates replaced by chlorides.

$Ca(NO_3)_2$	4 part
KH_2PO_4	1
KNO_3	1
$MgSO_4$	1
Fe_2Cl_6	trace

$CaCl_2$	4
KH_2PO_4	1
KCl	1
$MgSO_4$	1
Fe_2Cl_6	trace

III. Phosphate replaced by chloride. IV. Potassium replaced by sodium.

$Ca(NO_3)_2$	4
KNO_3	1
$MgSO_4$	1
KCl	1
Fe_2Cl_6	trace

$Ca(NO_3)_2$	4
NaH_2PO_4	1
$NaNO_3$	1
$MgSO_4$	1
Fe_2Cl_6	trace

V. Calcium replaced by sodium.

$NaNO_3$	4
KH_2PO_4	1
KNO_3	1
$MgSO_4$	1
Fe_2Cl_6	trace

VI. Magnesium replaced by sodium.

$Ca(NO_3)_2$	4
KH_2PO_4	1
KNO_3	1
$NaSO_4$	1
Fe_2Cl_6	trace

VII. Cane sugar.

The solutions are made up to 0.58 per cent except the full nutrient and the cane sugar solutions which are made up to 0.5 per cent. The solutions are made with sterilized distilled water, but the spores are not sterilized except for the sugar solution. It is possible to sterilize the spores of *Osmunda* without injuring their vitality. The method used is as follows. The spores are dipped into absolute alcohol for one minute, then immediately transferred to the sterilized distilled water which is kept in the sterilized Petri-dish. The spores are transferred to the sugar solution (also previously sterilized) by the sterilized pipette. Two weeks later, the culture is examined and the small prothallia are found at the bottom of the culture. The spores which have been treated with the alcohol, cannot remain floating on the surface of the nutrient solution, whereas those which are not treated, are able to remain on the surface of the solution and able to develop into the matured prothallia. There is a great difference in the growth condition between the prothallia which are grown on or in the nutrient solution.

Transpiration is prohibited if they are allowed to grow under the submerged condition, and the transpiration is one of the important factors in the sex development in fungi, and algae (cf. KLEBS, 1904).

The prothallia in the sugar solution are thus grown entirely under a submerged condition, though the antheridia are formed normally (see Table XVI and XVII). In the latter part of the experiment the contamination of species of yeast and *Mucor* is noted, though it was very slight and the culture remained clear till the end of the experiment.

The spores were sown on May 14th. Each culture contained 45 c.c. of the solution. The germination began as early as five days after sowing in almost all cultures. They were kept in the laboratory whose temperature in May was 17°C to 25°C and during the hottest months in summer reached over 32°C. The effects of the different nutrient solutions on the development of the prothallia are manifested in the very early stage. On May 25th, (twelve days old) the following observation was made. (1) The growth is very weak and the rhizoids are very short in Solution V. (2) The prothallia in Solution VI (*-Mg*) have long rhizoids, but the growth of thallus is not so good as those in Solution II, III, and IV. (3) No marked difference in growth is observed in Solution II (*-N*), III (*-P*), and IV (*-K*). (4) The growth is slightly better in the full nutrient than the above three.

The condition on June 2nd (nineteen days old) was as follows. (1) Those in solution V are very poor. All dwarfed, and some adventitive growth began. (2) Those in Solution III are better than those in Solution V. The prothallia are more or less regularly shaped, but not notched (no meristem differentiated). (3) Those of Solution II and III grew more or less in filamentous form. (4) The growth in the full nutrient solution is practically the same as that in Solution II and III.

In order to insure the purity of the solution and to compare with the other, on July 10th, one of the duplicate cultures of each solution was replaced by the freshly prepared 0.7 per cent solution of the same constitutions respectively. The condition of cultures at the 46th day was summarized as follows:

No.	Sol.	Conc.	Condition	Antheridia	Arche-gonia
1	II (-N)	0.58	Pale green, abnormal starch.	many	—
7	II (-N)	0.58— 0.7*	Greener than 1, abnorm. starch.	few	—
2	III (-P)	0.58	Green, filamentous, ameristic.	many	—
8	III (-P)	0.58— 0.7*	Green, filamentous, ameristic.	very few	—
3	V (-Ca)	0.58	Dwarf prothallia, branched ameristic.	—	—
4	V (-Ca)	0.58— 0.7*	Almost all of them killed.	—	—
5	VI (-Mg)	0.58	Green, ameristic, normal starch.	very few	—
6	VI (-Mg)	0.58— 0.7*	Green, ameristic, normal starch.	—	—
9	IV (-K)	0.58	Green, ameristic, normal starch.	few	—
10	IV (-K)	0.58— 0.7*	Green, branched irregularly.	few	—
12	Cane sugar	0.5	Pale green, dwarf, abnorm. starch.	many	—
11	KNOP'S	0.5	Deep green, more or less filamentous.	few	—

There are more or less distinct different forms are recognized in the prothallia of different cultures. For convenience's sake they may be grouped under the following four types, namely;

(1) Filamentous, comprising two or three rows of cells; long stretched ribbon-like or filamentous in shape. Those which are grown in the Cultures 1, 7, 2, 8, and 11 came under this type (Pl. X, fig. 1). This type occurs also in overcrowded sowing, grown under weak light, and in darkness. A good example of that is seen in *Ceratopteris* grown in absolute darkness (NAGAI, 1913, p. 289).

KNY (1872) observed the linear arrangement of the chlorophyll bodies in the cells of the prothallia of *Osmunda regalis*. The similar appearance is well observed in the Pl. X, fig. 1 (compare KNY's figure Tafel III, fig. 9).

(2) More or less elongated spoon-shaped. This is an intermediate form between normally heart-shaped, meristic and the filamentous, ameristic prothallia. Those in Culture 5 and 6 belong to this type (Pl. X, fig. 4).

(3) Irregularly branched. This type of prothallia belong to neither of the above two. The marginal cells branched out more or less adventitiously.

* Since July 10th in 0.7% solution.

An example is seen in Culture 3 (Pl. X. fig. 3).

(4) Normally heart-shaped, meristic prothallia. Naturally these groupings are approximate and arbitrary, many intermediate forms can be found. It is evident, however, that the concentration and the different kinds of nutrient solutions are able to modify the growth of prothallia from the normal type to diverse anomalous forms.

Further, the influence of the different solution on the sex development is observable. The measurement of the number of antheridia produced in the different cultures are given in the Table XVI and XVII.

Table XVI. *Osmunda regalis* var. *japonica*.

The influence of the different nutrient solutions (observed July 28th, 76 days after sowing).

Cult. No.	Sol. No.	Conc.	Total prothallia	Sterile prothallia	Total antheridia	Antheridia per proth.	Starch content
1	II (-N)	0.58	30	?	41	1.45	abnormal
7	II (-N)	0.58—0.7	50	25	36	0.72	
2	III (-P)	0.58	50	13	65	1.30	normal
8	III (-P)	0.58—0.7	—*	—*	—*	—*	
3	V (-Ca)	0.58	?	?	1	—	normal
4	V (-Ca)	0.58—0.7	—	—	—	—	
5	VI (-Mg)	0.58	100	100	0	0	normal
6	VI (-Mg)	0.58—0.7	119	119	0	0	
9	IV (-K)	0.58	50	43	7	0.14	normal
10	IV (-K)	0.58—0.7	43	?	7	0.16	
12	Cane sugar	0.5	24	?	?	36	abnormal
11	KNO ₃ 's	0.58—	50	19	106	2.12	normal

not observed.

Table XVII. *Osumuda regalis* var. *japonica*.

Same as Table XVI, observed Aug. 20th (99 days).

Cult. No.	Sol. No.	Conc.	Total prothallia	Sterile prothallia	Total antheridia	Antheridia average per proth.
1	II (-N)	0.58	68	18	70	1.08
7	II (-N)	0.58— 0.7	46	10	41	0.89
2	III (-P)	0.58	57	14	75	0.43
8	III (-P)	0.58— 0.7	47	28	20	0.90
3	V (-Ca)	0.58	47	40	6	0.12
4	V (-Ca)	0.58— 0.7	dead	—	—	—
5	VI (-Mg)	0.58	59	58	1	0.02
6	VI (-Mg)	0.58— 0.7	54	54	0	0
9	IV (-K)	0.58	49	39	12	0.24
10	IV (-K)	0.58— 0.7	54	39	17	0.31
12	Cane sugar	0.5	56	0	56	1.12
11	Knop	0.5—	88	22	266	3.32

The culture with cane sugar and with Solution II come next to the control culture in respect to the number of antheridia produced, showing that the slight amount of nitrogen is quite sufficient for the formation of antheridia. Number of antheridia is less in the series of cultures whose solutions have been changed. This is due perhaps to the fact that the renewed solutions are purer in respect to the elements replaced, and the raised concentration (made twice as strong as the original) might accelerated the injurious effect due to the absence of the specific nutrient mineral salt. Two phases can be considered in the action of different nutrient solutions on the prothallia. One is the direct effect of certain nutrient mineral salt which is in lacking and the other is the effect of the balance of the nutrient elements especially with reference to sodium, calcium, and magnesium.

The prothallia grown under a deficiency of nitrogen, cannot perform full growth, though capable of producing the antheridia. Whereas with those which are grown under magnesium hunger, the growth is not much

hindered but the development of sexual organs is almost completely suppressed. In normal KNOR's solution, the vegetative growth of the prothallia of that extent as does in magnesium hunger easily brings about the antheridia formation, but in the case with Solution VI no antheridia is formed. It suggests that magnesium is essential to the antheridia formation in *Osmunda*.

In the cells of prothallia grown in Solution II, starch is accumulated in abnormal quantity. The normal green color of the chlorophyll is changed to pale yellowish green. A similar condition is found also in the sugar solution. The accumulation is more pronounced in the latter. The chlorophyll bodies are entirely filled with starch grains. These prothallia do not reach more than twenty cells but the antheridia are well produced and the number of sterile individual in the culture is very small (Table XVI).

The deficiency in phosphate (Solution III) and potassium salt (Solution IV) seem to play less important role in the antheridia formation, thus the prothallia grown under such conditions, are able to form antheridia in a considerable amount and no abnormality in starch content in the chlorophyll bodies is observable. On the other hand, the effect of calcium hunger (Solution V) is somewhat pronounced. The prothallia in Solution V are dwarfed. Many cells die early. The sign of injury is manifested already nineteen days after the spores are sown. In the refreshed solution, the prothallia are killed entirely and the brown colored substance is markedly produced in the dead cells. Few individuals survived in the unrefreshed solution, but the growth was extremely unhealthy. The injurious effect of this solution may not due only to the absence of calcium but also to the excess of sodium salts in the solution which might have exerted a deleterious action. In any culture, no archegonia is found; this gives a further evidence for that much more favorable growth condition is necessary for the development of archegonia than that of antheridia.

III. On the Osmotic Relation to Sex.

The recent investigations of SPRECHER (1913) and TOURNOIS (1914) on *Cannabis*, *Rumex*, and *Humulus* bring out the problem of the difference in

the osmotic pressure and its relation to sex in these dioecious plants. SPRECHER finds that the average osmotic pressure of the sap from the male plant of *Cannabis sativa* is 10.578 atmosphere whereas that of the female plant is 10.104; for *Rumex acetosa*, the difference is 7.67 to 7.21. He thinks, however, that such a difference in the osmotic pressure of the extracted saps from the male and female plants are not due to the difference in sex but to that of the stage of development of the plants. For, the male plants mature much earlier than the female, thus the measurement of the pressure at the same period would not give the pressure of the sap of the nearly the same stage of their development. He states, "le difference de pression osmotique entre plantes mâles et femelles est simplement une question de développement" (p. 341). Though TOURNOIS holds the view that the difference in the osmotic pressure in *Humulus* is directly related to the sex of the plant. "Les variations sexuelles semblent dépendre des variations de la pression osmotique." The decrease in the osmotic pressure in the male plant can determine the appearance of the female flower or the female organs. The rise in the osmotic pressure in the female plant, though very rare, decides the appearance of the male organs or plant from the females.

These accounts in view, the measurement of the osmotic pressure of the cells of the prothallia is made. The plasmolytic method is employed. It is found that the osmotic pressure of the prothallial cells is very variable according to the individual prothallium as well as in the different parts of a single prothallium. It is clear, however, that the osmotic pressure of the cells at the region where the archegonia are formed is always higher than that of the remaining part where the antheridia are abundantly formed. It seems highly probable that this difference is simply due to the age of the cells, for the cells of the archegonia-bearing portion are comparatively younger than the rest. The cells of the former portion rest near the meristem whose osmotic pressure must be higher than the older cells. It may be, therefore, that the osmotic pressure of the cells that give rise to archegonia is higher than that of those which give rise to antheridia.

Further, the osmotic pressure of the prothallia is higher in those which are grown in the nutrient solution of the higher concentration than that of those which are grown in the lower concentration. In another words,

the cells of prothallia exert the osmotic autoregulation to the external media. The threshold concentrations are determined by the cells of the lowest turgescence in each prothallium so that the osmotic pressure of the cells at the archegonia-bearing region should be much higher than that so obtained.

Table XVIII. *Osmunda regalis* var. *japonica*.

The average osmotic pressure of the prothallial cells.

Conc. of media	Media	Threshold conc. <i>Na Cl</i>	t°	i^{**}	O. P. at 20°C
1 % Knop	sand	N 0.1830	17°C	1.8101	7.473 atm.
0.5 "	liquid	N 0.1290	19°C	1.8303	5.340 "
0.35 "	liquid	N 0.1290	19.5°C	1.8303	5.340 "
0.25 "	sand	N 0.1034	17°C	1.8142	4.280 "
0.025 "	liquid	N 0.1105	17°C	1.8395	4.813 "

Table XIX. *Asplenium Nidus*.

The average osmotic pressure of the prothallial cells.

Conc. of media	Media	Threshold conc. <i>Na Cl</i>	t	i	O. P. at 20°C
0.7 % Knop	liquid	N 0.1406	21.5°C	1.826	5.808 atm.
0.35 "	"	N 0.1375	20.5°C	1.827	5.688 "
0.175 "	"	N 0.1314	21.5°C	1.828	5.526 "

The difference in the osmotic pressure in the two species grown in the same concentration is determined by a parallel test.

	Conc.	Threshold conc. <i>Na Cl</i>	t	i	O. P. at 20°C
<i>Asplenium Nidus</i>	0.35 %	N 0.1285	20.5°C	1.827	5.688 atm.
<i>Osmunda regalis</i>	0.35 %	N 0.1375	21.5°C	1.8266	5.617 "

t = room temperature.

i = coef. of dissociation.

It shows that the osmotic pressure in *Osmunda* is practically the same as that of *Asplenium*. The highest osmotic pressure is found in the cells grown in the highest concentration and the difference between the highest and the lowest is more than 3.19 atmosphere at 20°C. Considering that the archegonia are not formed in the culture of high concentration as well as in the low in *Osmunda* (between 0.35—0.175%), there may exist some relation between the archegonia formation and the specific osmotic pressure. In the case with *Asplenium*, the archegonia is formed from 0.175 per cent up to 0.7 per cent KNOP's solution, though the optimum concentration is also found in 0.35 per cent as in the case with *Osmunda*. The osmotic pressures of the cells grown in that concentration is 5.647 atmosphere for *Osmunda* and 5.688 for *Asplenium*. Therefore the osmotic pressure lying somewhere at 5.6 atmosphere at 20°C may be considered as the optimum osmotic pressure for the archegonia formation for the prothallia of the experimented two species. The antheridia are able to be formed from 7.4 to 4.2 atmospheres which are the two extremes of the studied concentration in the case with *Osmunda*.

In view of the data at hand, it is far from satisfactory to draw any conclusion from the sexual difference and its relation to the osmotic pressure in fern prothallia. The assumption seems improbable that the permeability of the plasmic membrane, with its extreme susceptibility to external influences and its highly complex physiological, autoregulatory changes, cannot exert an influence upon the difference in osmotic phenomena of the cell which is regarded as solely due to the difference in sexuality.

IV. On the Starch Accumulation.

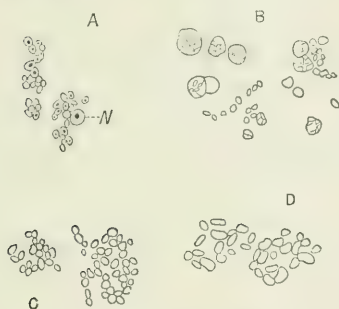
The attention has already been made to the abnormal accumulation of starch in the cells grown in the nutrient solution lacking in nitrates and in sugar solution. The fact is noted by PRANTL (1881), REED (1907), and the writer (1913) in the different species of ferns. The prothallia of *Osmunda* grown in cane sugar (0.5 per cent), in the nutrient solution without nitrate, and that of *Asplenium* grown in the distilled water showed marked starch accumulation in the chlorophyll bodies (see Text-fig. 7). But those grown in the nutrient solutions lacking in phosphate, calcium,

magnesium, and potassium respectively did not show any abnormality. This suggests that the phenomena are in some way connected with the deficiency in nitrogen. In order to ascertain whether or not the nitrogen hunger is actually one of the causes of starch accumulation, the following experiment is conducted.

The prothallia which are rich in starch and have been grown in Solution II (0.5 per cent without nitrates) are transferred to 0.1 per cent of the different kinds of nitrates and ammonium salt solutions. Soon after the prothallia are supplied by nitrogen, the accumulated starch begins to diminish. It is found, however, that the ammonium salts and asparagin solutions are injurious to the cells, being many prothallia are killed within a short time after they have been transferred. This is perhaps due to the alkalinity of the solution. Whereas the nitrate solutions are not at all injurious and they gave a new start for growth and the formation of antheridia. After two weeks, the cells in all cultures recovered their normality and the abnormal quantity of starch is diminished. The conditions of cells at the different periods are tabulated here:

Solution	Previous cond.	After 6 days	After 10 days
$CH_3(NH_2)$, CO_2H CH_2 , CO , NH_2	Abnormal starch, pale yellowish green.	Most of them died, fungus infection.	Killed.
$(NH_4)NO_3$	do	Pale yellow, fungus infection.	Many died, starch normal, slight new growth, many died.
NH_4Cl	do	Many died, fungus infection. Slightly new growth, fresh green color.	Starch normal, new growth and new antheridia.
$NaNO_3$	do	New growth, many still abnormal starch content.	Pale green, almost normal with few exceptions.
KNO_3	do	New growth.	Very healthy, green, new growth. Normal starch except few abnorm.
$Ca(NO_3)_2$	do	New growth, starch content normal in many individuals.	Healthy, normal starch with few exceptions.
Knop's	do	Green, starch cont. normal. New antheridia, and new growth.	Very healthy, strong new growth, normal starch with few exceptions.
Control	do	No change	No change

Whether or not the abnormal accumulation of starch is chiefly due to the inefficiency in the dislocation of starch formed in the chlorophyll is studied in the following experiment. The prothallia which have long been grown in the nutrient solution lacking in available nitrogen are placed in the dark together with the parallel culture of control which have been grown normally in 0.1 per cent KNOP's solution. At the end of two weeks it is found that the starch is still abundantly present in the cells grown in the solution lacking in nitrates. Whereas in the cells of the control culture, even ten days after the light was excluded, no starch was tested by potassium iodide iodine solution (IKI). The mortality of the cells in both cultures is tested by plasmolysis. They are placed in 20 per cent *Na Cl* solution and they are immediately strongly plasmolysed, showing that they are still alive in spite of their extremely unhealthy appearance.



Text-fig. 7. A-B. *Osmunda regalis* var. *japonica*. A. Normal starch in the chlorophyll bodies. N. Nucleus, 35 days in Knop 0.1 per cent formally grown in Knop without nitrate. $\times 113$. B. Abnormal chlorophyll bodies filled with starch, grown in Knop without nitrate. $\times 113$. C-D. *Asplenium nidus*. C. Normal starch. Knop 0.35 per cent. $\times 260$. D. Chlorophyll bodies, grown in distilled waters $\times 260$.

The above fact shows, if not conclusively, that the normal catabolic processes of the photosynthetic products are prohibited or at least strongly weakened by the ill feeding, thus the cells still contain abundant starch even when they are allowed to stand for two weeks in the absence of light. Presumably a normal supply of nitrogen is necessary for the realization of the normal function of cell activity to dislocate the starch.

NATHANSOHN (1910, p. 96) states the evidence of the plasmolytic method of acceleration of physiology of assimilation. The starch-freed

leaves of mosses are able to synthesize starch by treating them with a strong solution of cane sugar or some inorganic salt solutions which cause plasmolysis. Recently LUNDEGARDH (1913) studied the physiological conditions of

starch \rightleftharpoons oil, and starch \rightleftharpoons sugar relations in many oily and starchy seeds as well as in the green leaves. He has shown the elevation of concentration of cell sap either by drying or plasmolysis can change the equilibrium, though negative to NATHANSON'S statement. He assumes that the action of plasmolysis is such a nature as to change the permeability of the surface layer ("Hautschicht") rather than the concentration of the cell content (p. 450). However, further studies are much required.

V. Discussion.

The cytological evidences which support strongly the view of progamic and syngamic determination of sex in animals are decidedly negative in plants. The only literature at the writer disposal is the work by STEVENS (1912). In the heterostylous plants, *Eragrostis* and *Houstonia*, the investigator found that the chromosomes of the short-styled form have a diameter nearly twice as great as do of the long-styled form (cf. his fig. 14, 15 with 16 and 17). "The "central" chromosome is apparently considerably larger in one of the daughter nuclei of the heterotypic mitosis than is its synaptic mate in the sister nucleus," and "it bears a striking resemblance, however, to the condition found in the sperm mother cells of *Lygaeus* and other insects in which there is an "x" chromosome which has as a synaptic mate a smaller "y" chromosome." However, the evidence is not convincing enough to extend the chromosome theory of sex determination in plants. Many other investigators fail to discover any morphological difference in the chromosomes of the hermaphroditic and dioecious plants.

Inasmuch as the sex element is morphologically indistinguishable in the nuclear substances of the plant cells, the problem of sex determination cannot be attacked from the cytological investigations. Light must be given from experimental hybridization and physiological studies. Very important evidence in regard to the predetermination of sex is that given by BLAKESLEE (1904, 1906) on *Phycomyces* and other genera in Mucorineae. He has shown that the zygospore is formed only when the two different strains of mycelia plus and minus meet. Plus mycelium meeting with plus is never able to produce zygospore, neither the neutral with neutral nor minus with minus

mycelia. The neutral mycelium is, however, able to produce plus and minus mycelia by the asexual reproduction, thus it can be considered as bisexual. Further evidence is given by BURGEFF (1912) who has shown that the artificial mixing of the plasms of the two strains is able to change the sexuality of the mycelium thus mixed. Whereas the plus mycelium is never able to produce minus mycelium nor vice versa, but the mycelium which contains the plasms of the two by artificial grafting, is able to produce both kinds plus and minus, thus he verified BLACKESLEE's idea of the bisexual nature of the neutral mycelium. The presence of plus and minus strains is extended to *Glomerella* by EDGERTON (1914). His investigations show that the fusion of the two strains brings about the formation of ascus on the boundary lines where they come together in a plate, and it is proved to be that the both strains are present not only in a single perithecium but that they also present in a single ascus, for, they are found in the colonies developed from spores that formed in one of the colonies from a single ascus.

HÄBERLANDT (1869) found in hemp that sex is determined in the seed of the parental plant and that the nutrition, the time of sowing seed, and the like have no influence on the sex proportion of the progeny. Likewise HEYER (1884) arrived at the conclusion that the influence of external conditions do not change the sex proportion in hemp. Among 40,000 individuals under examination, he found the sex proportion as 100 males to 114.93 females. FISCH (1887) gives the following proportion, 100 males to 154.23 females. He believes that the sex proportion in hemp is constant at least in the race which he worked on. The deviation from this average never exceeds more than 5.5 per cent. The germination of the seeds or the development of the plants have no influence on the above proportion.

SPRECHER (1913) came to the conclusion that the sex proportion in *Cannabis* and *Rumex* is independent of manuring. The size of the seeds in *Rumex* has no influence in the sex proportion. He finds the proportion in *Rumex* 100 male to 241 females, and in *Cannabis* 100 male to 112 females. Further he finds that the osmotic pressure and the mineral and the organic matters contained in the extracted juice from the male and the female plant is different. In regard to the osmotic pressure, reference has already been made

in Section III. There is 10 gramme per litre excess dry matter found in the extracted sap from the male plants than that found in the sap from the female plants. But the ash content of the sap from the male is less than that of the female, and reversely, the organic matter is 12 gramme per litre more in the sap from the male plant than that from the female plants. He deducts, however, that these differences are not due to the difference in sex but that of the stages of development of the plants. In his own words, "Les substances organiques s'accumulant avec le développement maximum de la plante, les mâles seraient donc à un stade de développement plus avancé que les femelles et la différence entre la pression osmotique des deux sexes ne constitue nullement une différence essentielle, mais seulement temporaire, due à des degrés divers de développement. Deux individus ayant le même nombre de jours d'existence ne sont pas forcément du même âge au point de vue physiologique. C'est surtout le cas ici, où il s'agit de plantes mâles et femelles; la plante mâle, ayant terminé le cycle de son développement plus tôt que la femelle, est par conséquent plus avancée au moment de la floraison" (p. 351). COOK (1914) observed also the sexual inequality in the development. While the female plants grow vigorously, the male plants cease their life cycle even grown under the same conditions. The marked difference in the behavior of the two sexes of the same plant suggested to the investigator the idea of a sex-limited environmental character and "certainly there is no general reason or analogy for believing that sex alone would explain so great a difference of behavior under the same condition" (p. 203). According to WESTER (1914), CIESLESKI found that in *Cannabis* and *Spinacia* the sex is determined by age of the pollen at the time of fertilization. When fertilized with the younger pollen, he is said to have obtained the higher percentage of male plants and when plants fertilized with the older pollen, the higher percentage of female plants in the progeny.

Another set of data is also at hand which seems to show the contrary evidence, namely that the environmental influences are effective. LEYDECKER and BLOMEYER find that in *Cannabis* the weight of the seed and the time of harvest have no influence on the sex proportion of the progeny but the rich soil tends to increase female individuals and the poor

soil tends to give male plants in greater proportion. MOLLIARD (1898) believes that the environmental influences are effective on the sex determination in hemp but the chemical nature of the soil, the humidity, and the temperature are not the factors, but the intensity of the light is effective. But his result was not verified by STRASBURGER (1900).

LAURENT (1904) finds in *Spinacia* that the manuring has a direct influence on the sex proportion. An increase in the males is found by manuring nitrate and lime, whereas the application of potassium or acid phosphate tends to increase the females. Such influences are not only limited to the mother plants but also extend to the offspring obtained from the treated plants. The seeds from the plants manured by nitrate give more females than males, whereas the seeds obtained from the plants manured by the potassium, phosphate or calcium give the contrary.

DACKNOWSKI (1907) was able to show that the sex development in *Marchantia polymorpha* are responsible to the external influences. The high intensity of light and extended exposure to the same are the necessary conditions for the development of the reproductive organs, first the male and later the female organs appear. The same relation holds good also with the blue and red ray of light. If the thallus is allowed to grow under high humidity with weak light, the development of the sexual organs is entirely suppressed. Transferring the thallus from the atmosphere to the water or to weak KNOR'S solution (0.1 per cent), the formation of gammae and the sexual organs are also suppressed. The influence of poor nutrition, crowded growth of thalli and the combinations of these conditions are seemingly unimportant. The sex proportion in a culture of 173 plants, grown in a high intensity of light and humidity for three months, was 85 per cent male to 3 per cent female. He holds the view, however, that the sex in *Marchantia* is determined already in the gammae and the influence of strong light accelerates only the development of antheridia on the thalli which are developed from the male gammae.

A somewhat similar view is held by TOURNOIS (1914) who made an extensive study on sex in the species of *Humulus*. He was able to show that the external conditions, particularly the humidity of atmosphere and the intensity of light are able to transfer the male plant to functional monoecious plant

("fonctionnellement monoïques") or to the exclusive female plant ("exclusive-ment femelles"). Further, the male organs in the male flowers, can be "substituted" or "superposed" by the stigma or sterile carpels. He believes that these modifications are due to the change in the osmotic pressure of the plant or the organs. The higher or lower rate of transpiration increase or decreases the osmotic pressure of the plants under subjection to these causes, and the decrease in the osmotic pressure in the male plant can determine the appearance of the female flower or the female organs. The rise in the osmotic pressure in the female plants, though very rare, decides the appearance of the male organs or the plant from the females. He finds, however, that the transformation of sex is never definite and the initial sex reappears when the modified plants are replaced under normal growth conditions. He comes to the conclusion that the sex in *Humulus* is rested in the "preponderant" factors in the particular constitution of the ^{org.}

The extensive Mendelian studies led CORRENS (1913) to the view that the sex is characteristically hermaphroditic in its genotypic constitution, and that the suppression of the male part in "Anlage" or in the characteristic gives rise to females, and that the suppression of femaleness gives rise to males. "Beide sind, ihren Anlagen nach, eigentlich Zwitter." "Die Geschlechtsbestimmung kann nicht darin bestehen, dass dem einen Individuum männliche, dem andern weibliche Anlagen zugeteilt werden. Sie muss vielmehr dadurch zustande kommen, dass nur ein Teil von den überhaupt entfaltbaren Merkmalen zum Erscheinen bestimmt wird, mag es sich nun um die direkte Förderung dieses einen oder um die Hemmung resp. Unterdrückung des andern Teiles handeln. Wird z. B. der männliche Teil der Anlagen oder Merkmale unterdrückt, so entsteht ein Weibchen, wird der weibliche unterdrückt, ein Männchen" (CORRENS 1913, p. 16). SHULL (1914) also holds the view of the hermaphroditic nature of sex. "The genotypic nucleus which is common to both the males and females of any species, contains in itself nearly all of the elements necessary to the production of both the male and the female of the species, and it is therefore to a large degree essentially hermaphroditic" (p. 297). Though he disfavours the statement that both sexes are possessed in each of them, because the expression

that the female possesses maleness or the male possesses femaleness is based "not upon what the two sexes possess, which is distinctive to each, but upon that which they possess in common," and in reality "that what they both possess in common is nearly all of that which they each possess, and that the additional element or elements which are required for the actual realization of the one or the other sex, are conceivably of relatively minor value" (p. 297—298).

It may be considered, therefore, that the determination of sex is in reality a modification of bisexuality. The modifier or determiner may rest on the peculiarity of the sex chromosomes (progam) or on the state of the inner complex of the germ plasm after fertilization (syngam) or else on the external influences on embryo (epigam); the circumstances which actually realize the one or the other sex may differ in different cases.

The works of PRANTL (1881), PERRIN (1908), BOODLE (1908), Miss WUIST (1910, 1913), and the writer (1913) seem to support strongly, together with the data given in this paper, the view that the fern prothallia of many species are bisexual in nature, and the realization of each sex most likely rests solely upon the external factors. The works of MARCHAL also show that the dioecious Bryophytes, such as *Bryum coespitium*, *Mnium hornum*, and *Bryum argenteum*, are potentially bisexual, though the sex differentiation is initiated at the time of sporogenesis.

The gametophytes of the ferns known as prothallia are able to perform the independent life from their sporophyte and this makes quite a different relation to the environmental influences from those which exist in the Phanerogams such as *Humulus* or *Cannabis* in which the gametophyte is associated with the sporophyte, thus the relation to the external factors are much more complicated.

The prothallia of *Todea*, *Onoclea*, *Osmunda*, *Asplenium* and *Ceratopteris* all alike show that the development of male occurs even under unfavorable growth conditions but that of female requires better conditions. It may be that the male is a more phylogenetically primitive form than the female, thus the male appears even under the extreme conditions. The biological aspect of it seems also justifiable. The female should be well nourished so

as to produce strong offspring. The appearance of the females under unfavorable conditions in life seems to be useless, for they are exposed to the danger of extermination. Many investigations in the higher plants which have already been referred to also support the evidence gained in the fern prothallia. However, negative result is also reported. CORRENS (1905, 1908) finds in the gynomonoeious plant, *Satureia hortensis* that unfavorable nutrition (the weak illumination) increases the percentage of female flowers, and that favorable conditions (less number of seeds planted) on the contrary defers the admittance of almost pure female conditions of the plants. He concludes, thus, that the development of the hermaphrodite or the physiological female flowers is dependent on the nutrition, the richer nutrition for hermaphrodite, and the poorer nutrition for the female flowers.

It is shown clearly in the prothallia of *Asplenium Nidus* that the development of the male organ requires much less time than that which is required for the female organs and this seems to be universal in the plants of various kinds; namely in *Marchantia* (DACKNOWSKI, 1908), *Satureia* (CORRENS, 1908), *Cannabis* (SPRECHER, 1913; COOK, 1913) and *Humulus* (TOURNOIS 1914).

As already mentioned, since we assume that the sex determination (modification) is subject to external influences, if not in every case, the presence of maleness and femaleness as a "tendency" (compare CORRENS, 1913, p. 69; also STRASBURGER, 1910) or as a "potentiality" in the not-differentiated gametophytic cells of prothallia is a necessary conclusion. The sex potentialities may be, then, considered to be involved in the "specific structure" (in the sense of KLEBS, 1913) of the plasm, and the factors which release the potentialities to realize the one sex or the other, or both are the "internal conditions" which are dependent upon "external conditions"—especially the nutrition. The external conditions modify the "internal conditions" of the cell and through these, the initial change in the "specific structure" occurs, thus the sex is realized.

The specific localisation of the archegonia on the prothallia is suggestive in this regard. The exact nature of the inner complex is unknown, but the archegonia is peculiarly limited to develop at certain cells near the meristem, and it seems probable that the "internal conditions" of these cells are such that the "potentialities" for femaleness is released into activity, whereas in

the cells of the other parts, their "internal conditions" are such that the potentialities for femaleness are unchanged, thus the archegonia would never be produced in these cells. It was suggested by the writer (1913) that the difference in nuclein and plastin of ZACHARLAS contained in these cells might be an important factor. The formation of meristem is only possible under favorable conditions, for example 0.175 per cent KNOR's solution for *Asplenium Nidus*. Then, this concentration becomes to be one of the external factors which determines the development of the archegonia in that fern prothallia.

The formative influence of the external conditions on the "internal conditions" seems specific to the individual cells but not alike to all the cells of the prothallia. The same factors act differently on the different parts of the prothallium. The marginal cells of a well grown prothallium of *Osmunda* are able to form the antheridia while some of the cells near the meristem of the same prothallium are developed into the archegonia instead of the antheridia. The cases with *Melandrium* and *Inachus*, the formative influence of parasitic organisms on the sex of the hosts are no doubt due to the change in the internal conditions caused by the infection of the parasites which ultimately give rise to the development of the sex which otherwise would never be realized.

It is too far fetched at present, however, to attempt anything further since that is beyond the reach of our exact information in regard to the real nature of the "tendencies" and the "potentialities" or anything so conveniently termed. Physiologically speaking, the Mendelian terminology so commonly used to-day, such as the "genes" and the "factors" and the like, has no great importance since it refers only to the *facts* acquired or those to be acquired, but not the *causes*. Even the term "stimulus", for example, as often used in the physiological sense, means to state merely the fact which in reality involves most complex physical and chemical processes in the living organism, and the phenomena like heredity and reproduction must involve the most complex inner changes that we can think of, of which we are at present utterly ignorant.

Summary.

1. The development of the antheridia and the archegonia in the prothallia of *Asplenium Nidus* and of *Osmunda regalis* var. *japonica* are dependent on the concentration of KNOR's solution by which they are grown. As a whole, the total number of antheridia as well as the average number per prothallium produced, decreases as the concentration decreases. In *Asplenium Nidus*, the number of the sterile prothallia increases as the concentration of the nutrient solution decreases. But in *Osmunda* no such relation is found.

2. In both experimented species, the archegonia are formed only above 0.175 per cent KNOR's solution. The best concentration for *Asplenium Nidus* is 0.175 per cent, and for *Osmunda regalis* var. *japonica*, 0.35 per cent. In *Osmunda*, the archegonia are not formed in the 0.7 per cent solution, but in *Asplenium*, they are formed in the same concentration.

3. In *Osmunda*, antheridia are possible to develop in 2.0 to 0.0175 per cent KNOR's solution as well as in the distilled water. In *Asplenium Nidus*, however, the concentration of nutrient solution is required to be above 0.0175 per cent for the formation of antheridia. Prothallia grown in a solution lower than that concentration are found to be almost completely sterile.

4. It is observed in many prothallia of *Asplenium Nidus* that both sexual organs appear only successively but not simultaneously, consequently they appear to be dioecious.

5. The prothallia of *Osmunda regalis* var. *japonica* grown in the nutrient solution which lacks in calcium or magnesium salt remain almost completely sterile.

6. The osmotic pressure of the cells of prothallia of both species is variable according to the strength of the nutrient solution upon which they are grown either by sand or by liquid culture. The highest osmotic pressure is found in the cells which are grown in the highest concentration within the range of the experimented concentrations. The osmotic pressure decreases as the concentration of the nutrient solution decreases.

7. Starch is accumulated abnormally in the chlorophyll bodies of the

prothallia of *Osmunda* which are grown under nitrogen hunger condition. Normality is soon recovered if they are supplied with the weak solutions of various ammonium salts and nitrates.

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EXPLANATION OF PLATE

PLATE X.

Photomicrographs of the prothallia of *Osmunda regalis* var. *japonica* grown in the different nutrient solutions (taken seventy seven days after sowing). ca. $\times 65$.

Fig. 1. The nutrient solution without nitrate (Sol. I).

Fig. 2. " " " " magnesium salt (Sol. VI).

Fig. 3. " " " " calcium salt (Sol. V).

Fig. 4. " " " " potassium salt (Sol. IV).

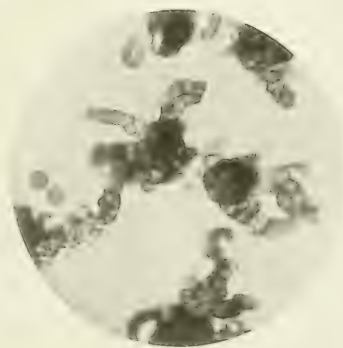
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Genetical Studies on *Oxalis*.

By

Sigeroku Nohara.

With Plate XI.

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I. Introduction.

Several forms of the common *Oxalis* are met with in Tōkyō and its vicinity. Some of them have a purple eye on the corolla, while others have none; some have purple leaves, others have not. As will be shown later, some taxonomists seem to include them in one species, while others consider each of them as a distinct one. The diversity of these forms attracted my attention and led me to investigate their relationship from the genetical

point of view. Although I do not intend to study them taxonomically my investigation will throw some light on the taxonomic value of these forms.

The work was commenced in the summer of 1909 and has been continued until now. The chief results so far obtained are reported in the present paper.

II. Material and Methods.

The material I used was mainly collected in Tōkyō and its vicinity, but some was brought from as far as Kamakura and Nagoya. To begin with, I classified all the individuals which were collected from those localities into 4 groups according to their most apparent distinguishable characters, and designated them for my own convenience as Type I, II, III and IV respectively. The following table will explain the distinction between these 4 types :

Table I.¹

Type \ Parts	Corolla	Leaves
I	Lemon yellow throughout	Almost quite green
II	Yellow throughout	Purple green
III	Yellow, but each petal has a dilute purple bar near the throat, so that the corolla has the so-called eye.	Purple green
IV	Gamboge, but each petal has a deep purple bar near the throat, so that the corolla has a deep purple eye.	Dark purple green

As mentioned above, I and IV are the extreme forms with regard to the intensity of the purple color, while II and III are intermediate in this respect.

The purple color of the plants is due to the presence of purple anthocyan in the epidermal cells of the leaves or stems, though some

1. Plate XI will show the types and their characteristics.

colorless cells are also found there. In the corolla the purple cell-sap is contained most abundantly in the cells of the part, where the eye is to be formed. The yellow color of the petals is due to the presence of yellow chromo-plasts in the cells of the fundamental tissue.

For investigation I carried on pure culture as well as cross-testing. When the hybridization was to be made, the castration was performed one day before the opening of the flowers. For crossing it is sufficient to rub the stigmas of one flower with the open anthers of another. But when they are to be self-pollinated they may be left alone, only they must be protected against insects or strong winds.

III. Pure Culture.

The culture of $I \times I$ was continued to the 4th generation, that of $II \times II$ and $IV \times IV$ to the 3rd generation, and it was found that these types always bred true to themselves.

As a result of these experiments I consider each of the types I, II and IV to represent a distinct homozygote, but type III split in its 2nd generation and proved to be a heterozygote, as will be described in the following lines.

$III \times III$: The offspring from this combination were 130 in number and among them two forms appeared, which I shall call A and B.

The individuals of A-form, which were 96 in number, resemble their parents as a whole, but those of B-form, 34 in number, were quite different. The petals of the B-form which are a little smaller than those of II are yellow, and the leaves, which are also somewhat smaller, are green like those of I except in their margin, which is tinged with purple color as shown in Fig. 4.

I made the pedigree culture of B-form for two successive generations and it proved to be a distinct, pure type, which was thus extracted from type III.

We have already noticed that the proportionate number of individuals of the form A and B produced by self-pollination of the type III was respectively 96 : 34 or nearly 3 : 1, and it can be presumed that form III

was a heterozygote, that it separated by self-fertilization into two forms after the Mendelian rule, and that B-form is an extracted constant (or recessive) form, as far as the non-purple color in the petal is concerned. If this assumption is true, the question naturally arises, what is the other parent of the heterozygote type III.

When we examine closely the individuals of A-form we find among them some with petals of a deep purple color and the leaves just as in IV, and some others which are in this regard much nearer to the parent form. Among 96 purple individuals I selected 15, of which 5 were almost identical with type IV, and 10 nearly the same with III in all respects.

I continued the pedigree cultures of these individuals, which are now in the 3rd generation, and got the result shown in the following table.

Table II.

		Nos.	Numbers of individuals obtained by self-pollination	
III A	Plants identical with IV	1	92 like IV only.	
		2	163	..
		3	85	..
		4	130	..
		5	died	
	Plants identical with III	6	77 like III A	25 like III B
		7	35 ..	10 ..
		8	15 ..	13 ..
		9	81 ..	28 ..
		10	105 ..	33 ..
		11	141 ..	45 ..
		12	95 ..	42 ..
		13	53 (only purple ones)	
		14	80 like III A	81 ..
		15	71 ..	24 ..

As is shown in Table II, the 5 individuals which were taken to be identical with type IV gave offspring of the same type as their own, though No. 5 died before producing seeds. From this result we may conclude with great probability that these 5 individuals were homozygous.

The 10 individuals which were considered identical with type III gave offspring of two sorts, excepting No. 13 which proved to be homozygous. The individuals belonging to one of these two sorts resemble the form A, some of which have a deeper color than others; and those individuals belonging to the other sort resemble the form B, the numbers thus produced make the approximate ratio of 3:1 as shown in the table: that is to say the form A split into two forms (really 3) just as was the case with their parents (or III)

Based on the figures of the table we make the following calculation:

Individuals like form A	734 ¹
Individuals ,, B	253
Total number.....	987
The formula of standard error	\sqrt{npq}
$n=987$ $p=\frac{1}{4}$ $q=\frac{3}{4}$	
$\therefore \sigma=\sqrt{npq}=\sqrt{987 \times \frac{1}{4} \times \frac{3}{4}}=\pm 13.60377$	
Theoretical III A form	$987 \times \frac{3}{4}=740.25$
Empirical ,,	<u>734</u>
Difference	= 6.25
or	
Theoretical III B form	$987 \times \frac{1}{4}=256.75$
Empirical	<u>253.00</u>
Difference	= 6.25

The difference is therefore ± 6.25 and the standard error is ± 13.60377 , which is greater than the former and we see that the ratio of empirical numbers is reliable.

There are surely, among the form A, two sorts corresponding to the type IV and III respectively, probably making the ratio of 1:2, though it is very difficult to distinguish them exactly.

1. YULE, U. G.:—An Introduction to the theory of Statistics.

A similar case is met with in the cross-testing between types I and IV, of which a description will be given later on.

From the above mentioned experiments it can be concluded that III was a hybrid between the types IV and B-form which was extracted from III, and that by self-pollination it segregated into the two parental types and their hybrids in the ratio of 3:1 or probably 1:2:1, after the Mendelian Rule; so that the 2nd generation of III by self-pollination corresponds to the F_2 -generation of the hybrid $IV \times IIIB$.¹

Assuming the type III as a hybrid I identified the purple parent of this hybrid with the type IV of my series. I looked for any plant of such type as III B which would grow wild in the ground of our college, and I found some plants which are exactly identical with III B and which are rather common.

The reason I did not include this type in my series from the beginning is, because it had been considered as a fluctuational form of type I.

I took several individuals of this type, added them to my series as type V and preserved them to test their further behavior.

V \times V: Two of the individuals which were brought from fields were self-pollinated and some seeds were got from them. 55 individuals were raised, they all bred true to the type and showed that they were of the same type as III B. The 3rd generation was cultivated and found to breed true.

To sum up, from the above mentioned experiments we may say that the types I, II, IV and V are quite distinct as far as the characteristics in question are concerned, and that the type III is a hybrid between IV and V, so that the types of my series are reduced into I, II, IV, and V.

IV. Cross-Testing.

I have made hybrids between the above mentioned types of *Oxalis* following the combinations:

"I \times II, I \times IV, II \times IV, III \times V and their reciprocals"

1. III B represents the B-form which was extracted from type III by self-pollination.

1. CROSS BETWEEN THE TYPE I AND IV.

(A) I×IV: 16 F₂-individuals were obtained. They all resembled each other and showed the following characteristics:—Petal with a purple arch-shaped line on the yellow ground, forming an eye on the corolla. Purple color a little paler than that of IV. Leaves and stems purple, also paler than those of IV (see Fig. 6).

Experiments have shown that the hybrid is intermediate between two parents as to the color-intensity. I obtained some seeds from these hybrids by self-pollination, and from these I got 268 F₂-individuals, of which 199 were purple and 69 green, thus approximately in the ratio of 3:1.

$$\sigma = \sqrt{npq} = \sqrt{268 \times \frac{1}{4} \times \frac{3}{4}} = \pm 7.08872$$

$$\text{Theoretical green individuals} \quad 268 \times \frac{1}{4} = 67$$

$$\text{Empirical green individuals} \dots\dots\dots 69$$

$$\text{Difference} \dots\dots\dots 2$$

The above mentioned green individuals of F₂ are identical in every respect with the type I, and besides they were found to breed true, so it is clear that those individuals are extracted recessive plants as in the case of extracted green ones or V from the individuals of III×III.

As to the purple individuals we can distinguish two forms according to the nature of the color, and divide them into two sub-classes. The individuals of one sub-class are deeper colored than those of the other, and the former are identical with the type IV, while those of the other sub-class are identical with their parents, i.e. F₁-form.

The exact number of individuals of these two sub-classes, however, could not be determined on account of too great fluctuation, but evidently the deeper colored ones much exceed the other in number, and probably we can estimate their ratio as 2:1.

I got by self-pollination some seeds from 3 deeper colored individuals and 6 less colored ones. Those seeds gave rise to plants, which are shown in the following table:—

Table III.

	Nos. of F ₂ plants	Individuals in F ₃ generation	
Less purple colored	1	123 purples	45 greens
	2	21 ..	10 ..
	3	40 ..	14 ..
	4	105 ..	35 ..
	5	46 ..	13 ..
	6	22 ..	10 ..
Deep purple colored	7	137 purples	no greens
	8	62
	9	159

As shown in the table, the deeper colored individuals breed true, while the less colored ones give rise to two kinds of offspring, namely green as well as purple individuals. We may, therefore, conclude that we have in this generation two kinds of purples: heterozygous and homozygous.

As shown in the table the total number of offspring of the heterozygous purples in F₃ is 360 purples, 127 greens, and

$$\sigma = \pm \sqrt{npq} = \pm \sqrt{487 \times \frac{1}{4} \times \frac{3}{4}} = \pm 9.55575$$

Theoretical purples 365.25

Empirical purples 360.00

Difference 5.25

So that evidently the Mendelian law holds good here.

Again we could distinguish heterozygous purples from homozygous ones among the purples of the F₃ generation, though it is not so easy as in the case of IIIA already described.

From the above mentioned experiments we learn that:

a. The hybrid between I and IV is purple, the color-intensity being intermediate between that of its parents.

b. The individuals in the F₂ generation are of 3 different forms, namely: the deeper purples, dilute purples and greens, making the ratio

1:2:1 respectively, so that the form F_1 is split after the simple way of MENDEL's law.

c. The types I and IV are constant and quite distinct.

(B) $IV \times I$: I tested the behavior of the reciprocal hybrid with the above, i.e. $IV \times I$.

14 F_1 -individuals were obtained and they were in almost all respects identical with those of $I \times IV$. Of the 399 F_2 -individuals 294 were purple and 105 were green, i.e. approximately in the ratio of 3:1.

$$\sigma = \sqrt{399 \times \frac{1}{4} \times \frac{3}{4}} = \pm 8.64942$$

Theoretical green individuals 99.75

Empirical „ „ 105.00

Difference „ „ -5.25

Theoretical purple individuals 299.25

Empirical „ „ 294.00

Difference „ „ +5.25

The purples were also of 2 kinds, less deep and deeper colored ones, it was not always easy to distinguish them. The number of the former was, however, larger than that of the latter, probably making the ratio 2:1, just as was the case in the F_2 generation of $I \times IV$.

So we have, in the F_2 generation, three forms of individuals in the ratio 1:2:1, just as in $I \times IV$ in the same generation, as far as the color-intensity is concerned.

A further experiment of F_3 generation was accomplished as was done in the F_3 of $I \times IV$ and the result was found to be quite the same as in the case of $I \times IV$.

From the above mentioned experiment it is almost certain that two reciprocals of these hybrids are exactly of the same nature.

2. DOUBLE RECIPROCAL CROSS.

I tried to determine the nature of the double reciprocal cross in DE VRIES' sense, which consists of $I \times IV$ and $IV \times I$,¹ and obtained good

1. DE VRIES, H.: Über doppelreziproke Bastarde von *Oenothera biennis* L. and *O. muricata* L. Biologisches Centralblatt, Bd. XXXI, Nr. 4, 1911.

seeds from the cross, 198 individuals were raised, of which 156 were purples and 42 greens. Examining the characteristics of these 2 sets there was nothing particular found, but the purples and greens correspond to those of F_2 of either parent, i.e. $(I \times IV) \times (I \times IV)$ or $(IV \times I) \times (IV \times I)$ respectively.

The number of these 2 sets make the approximate ratio of 3:1.

$$\sigma = \pm \sqrt{198 \times \frac{1}{4} \times \frac{3}{4}} = \pm 6.09303$$

Theoretical	148.5	purples	49.5	greens
Empirical	156.0	„	42.0	„
Differences	-7.5	„	+7.5	„

The purples consist of 2 forms, i.e. deeper colored ones or the type of IV, and the less purple colored ones or that of the parents (either $I \times IV$ or $IV \times I$), probably making the ratio of 1:2.

There are therefore in this case three forms of offspring, i.e. purples, lighter colored purples, and greens, probably in the ratio 1:2:1 respectively, just as in the case of the second generation of the single hybrid.

I selected 3 individuals from each set of these 3 forms and obtained some seeds from them by self-pollination.

The greens (454 in number) and the deeper colored purples (more than 100 in number) were found on further cultivation to breed true.

Each lighter colored purple, on the contrary, was found to split into 2 forms, approximately in the ratio of 3:1 as follows:

99=	75 purple (deeper colored mixed with less colored)+	24 greens
218=	158	„ + 60 „
185=	138	„ + 47 „

The above experiments decidedly show that the nature of the products of the double reciprocal cross is quite the same as those of the self-fertilized F_1 of either $I \times IV$ or $IV \times I$.

3. THE IMPOSSIBILITY OF THE NEW FORM, SUCH AS THE GREEN LEAVED TYPE BUT WITH EYED COROLLA.

As is shown in Pl. XI, the corolla of type I is entirely yellow, and its

leaves are green, while the type IV has purple leaves and a purple eye in its corolla.

Let the type IV be represented by the formula $AABB$, and type I by $aabb$, so far as the colors of the petals and leaves are concerned. The F_1 individuals may be written $ABab$. In F_2 we have then

$$AABB + 2AABb + 2ABBa + 4ABab + 2Aabb + AAbb + 2BaaB + BBaa + aabb.$$

We consequently have 9 individuals with both purple leaves and corolla, 3 with purple corolla and green leaves, 3 with purple leaves and yellow corolla, and one with yellow corolla and green leaves. But in F_2 of $I \times IV$ or $IV \times I$ we have no such forms as purple eyed with green leaves, or purple ones without eyes among all the individuals, although 667 were cultivated.

From this result we may conclude that the eye-color in the corolla and the purple color in the leaves are due to one and the same factor, at least in this type (IV).

Again, that the eye-color and the leaf-color are due to one and the same factor is also proved by the splitting manner of forms in F_2 of the hybrid:

A eyes and leaf-color.

a the absence of the factor of the above

Aa and aA are therefore both parents

F_1 Aa

F_2 $AA + Aa + aA + aa$

This is really a monohybrid and it is clear that such a type as the green leaved with eye corolla is not to be expected

4. CROSS BETWEEN TYPES I AND II.

(A) $I \times II$: In the F_1 -generation of the hybrid $I \times II$, 38 in number, the leaves are more green than those of II, but more purple than those of I. It is remarkable that the margin of the leaves is much more conspicuously colored than those of II.

The color of the flower is yellow throughout, as was the case with that of II, there being no purple color, which was quite as was to be expected.

Of the F_2 -individuals, 31 in number 7 were identical with I, 16 identical with parents or with F_1 , and 8 identical with II, though the individuals of II-type are hybrids and not easily distinguishable.

The number of individuals of the three types above mentioned make the approximate ratio 1:2:1, though the number is too small to be relied on.

The cultivation of F_3 -generation was made. 56 F_3 -individuals from I-typed parents and 126 F_3 ones from II-typed parents proved to breed true. Of the individuals of the intermediate form, 51 in all, 15 were of I-type and the rest (36) were of 2 kinds, i.e. II-typed ones and the intermediate ones, the latter much exceeding the former in number.

From the above mentioned experiments we see that the behavior of the hybrid $I \times II$ follows the simple Mendelian rule.

(B) $II \times I$: The F_1 -individuals, 24 in number, present the same features as those of $I \times II$.

Although I did not further experiment with this reciprocal I am quite sure that it holds the same relation with the other, just as $I \times IV$ and its reciprocal do.

Based on the experiments we draw the same conclusion as in the case of $I \times IV$ and $IV \times I$, and we see that:—

- a. Type II is constant, showing a quite distinct strain.
- b. Both $I \times II$ and $II \times I$ are identical, and their form is intermediate between the parents in regard to the color-intensity.
- c. The hybrid between the non-eyed and non-eyed ones possesses the non-eyed corolla as might be expected.

5. CROSS BETWEEN TYPES II AND IV.

(A) $II \times IV$: The F_1 -individuals, 15 in number, were almost similar in all respects.

The flower of the individuals of this generation has an eye on the yellow corolla like that of $I \times IV$, while the leaves resemble those of $I \times IV$. In short, the nature of the color-intensity of the eye and leaves is intermediate between the two parents.

(B) $IV \times II$: Twenty individuals were obtained. Not only do they resemble each other, but also to $II \times IV$, so that both reciprocals are similar.

I tried the cultivation of the further generation and all the results of the experiments are summarized as follows:

	F_1	F_2	F_3
		9	133.....always II typed
II \times IV.....1		19	171 { 125.....IV typed+intermediate 46.....II typed only
		8	98.....IV typed
		13	56.....II typed
IV \times II.....1		24	51 { 37.....IV typed+intermediate. 14.....II typed
		11	131.....IV typed

From the above mentioned experiments we can draw the same conclusion regarding hybrid II \times IV and its reciprocal, as about several hybrids before mentioned.

6. CROSS BETWEEN TYPES III AND V.

By the test of the pedigree culture we found that III is an F_1 -form between IV and V, the presence of the purple color being dominant over its absence. It follows therefore that the hybrid between III and V corresponds to the back cross (IV \times V) \times V, and in this case we can expect the same number of purple-eyed and non-purple-eyed offspring, which was really proved. The results I obtained were:

$$\text{III} \times \text{V} \begin{cases} \text{eyed, 51 individuals.....} A \\ \text{non-eyed, 50 individuals.....} B \end{cases}$$

On further cultivation the form B has proved to breed true, while from A I obtained two forms, 123 purple-eyed and 45 non-eyed forms. It is clear that the numbers of these forms make an approximate ratio of 3:1. In this ratio the left side one would contain 2 distinct forms corresponding to IV and III in the ratio of 1:2, but I did not examine them closely.

From the experiments with III and V it was ascertained that:

a. The back cross with recessive produces the same number of dominants and recessives.

b. The assumption of III to be the hybrid form by the pedigree culture is correct.

V. Discussion.

Since the reappearance of MENDEL's work and the appearance of JOHANNSEN's "Pure-line theory" the opinion of naturalists on species or varieties has entirely changed, and they are now endeavoring to test plants and animals on the basis of the modern genetic principles.

According to the modern naturalists the relationships between species or varieties cannot be accurately known, and the determination of the species or varieties can hardly be relied upon in the strict sense, unless they are tested experimentally, because even the same species or varieties have a different appearance or structure, and vice versa, as we see in the well known experiments of BONNIER¹ and KLEBS.² When we therefore want to determine a species we should test it by cultivating it at least during 2 or 3 generations, under as near as possible the same conditions of environment, in order to make a just comparison.

The distinction of one species from another is therefore no easy task, and that of a subspecies or variety from another is exceedingly hard work. ALMQUIST's elementary species seem not to stand the test of long culture under more carefully controlled conditions such as SHULL employed.³

If I had determined various individuals without pedigree culture, I would have taken type III as a distinct one, and would not have known the existence of type V.

Thus the types I, II, IV and V were not only tested by pedigree culture, but also by cross-testing, so that I am positive that they are quite distinct biotypes. The experiments have shown me that the common *Oxalis* or the so-called *Oxalis corniculata* L. is a composite species, including at

1. BONNIER, M. G.: Recherches expérimentales sur l'adaptation des Plantes au climat alpin. Ann. des. sc. nat. VII, 20, 1804.

2. KLEBS, G.: Alternatives in the Development and Forms of Plants as a result of Environment. Proc. Royal Soc. Vol. 82, 1910.

3. SHULL, G. H.: Bursa Bursa-Pastoris and Bursa Heegeri, Biotypes and Hybrids. Wash. D. C. Pub. by Carnegie. Inst. 1909.

least four different biotypes, and in nature these four types and their hybrids grow together, so that those individuals make a continuous series in regard to the characters of flowers and leaves etc. For this reason it is very likely that these forms have been included in one species of *Oxalis corniculata* L.¹

In this case my type V seems to correspond to the typical one among them, i.e. *O. corniculata* L. But some taxonomists still keep the name of *O. stricta* L. as a distinct one, and in this case my type I seems to correspond to that.²

NAKAI does not take the nature of the stem or stipules for the distinction-character, but he lays stress upon the nature of the root-system. I do not, however, think the nature of the root-system to be the decisive character of this species, though my type I may be identical with NAKAI's *O. stricta* L., for my experiments with this type did not necessarily show the character as in NAKAI's note.

According to MAKINO³ there is a variety of the common *Oxalis* known as *Oxalis corniculata* L. var. *tropaeoloides* (Schlachter) Mak. and he thinks the existence of the intermediate form between the type and this variety to be possible; and the type IV of my series seems to agree with the variety just mentioned (Fig. 3.)

Although my Type II has not yet been investigated by any taxonomists it would be right to give it a special name, for it is a distinct type just as the type I, IV or V is a distinct one, and has its own name given by some taxonomists; otherwise all these types would be included under any one specific name, such as *Oxalis corniculata* L., as is done by some other botanists.

Up to the time of FOCKE⁴ the hybridization of any kind of *Oxalis* seems not to have been known, neither has any literature, except

1. MATSUMURA, J.: Index Plantarum Japonicarum, Vol. II, 1912.

2. ENGLER, u. PRANTL: Die natürlichen Pflanzenfamilien III. 4. NAKAI, T. Distinctive characteristics between *Oxalis corniculata* L. and *O. stricta* L. Bot. Mag. Vol. XXVII, Tokyo, 1913.

3. MAKINO, T. Observations on the Flora of Japan. Bot. Mag. Vol. XXVII, Tokyo, 1913.

4. FOCKE, W. O.: Die Pflanzennischlinge. Berlin, 1881.

one notice,¹ concerning the hybrids of this genus, at least of the wild forms, come to the knowledge of the present writer. But the feature of the purple color-inheritance in *Oxalis* very much resembles that of cotton investigated by LEAKE.²

The red color is due to the presence of anthocyan in his cotton-plants as well as in my *Oxalis* plants. The hybrid between his type 3, or 11 which is red, and the types in which the color is absent resembles that between IV and I in *Oxalis*. In both cases the presence of red is dominant, though the intensity of the red color is, as he says, greatly diminished.

LEAKE'S "type 3 \times type 4" corresponds to my "type IV \times type I," and the formation of the flower eye of the hybrid is due to the presence of the red sap upon the yellow-ground-color which is manifested in the eye of the parent flower.

The purple color in the flower and that in the leaves are due to the same one factor in type II, and such instances are rather common in plant-bodies, as in GREGORY'S experiments with *Primula* (full colored \times albino), for he says that in the full colors the color in the flowers and stems behaves as a single unit.³ LEAKE also seems to think, in his two types 3 and 11, that the coloration in the petals and in the remaining part of the plant originates from one factor. But this is not always the case; for example GREGORY points out, alluding to KEEBLE and PELLEW'S experiments and his own, that there are cases where the color-factor which is present in the stem, but not in the flower, is not one.

VI. Summary.

1. The materials used for the experiments were the types I, II, III, IV and V, and the distinctive characters of these materials were in the

1. BATESON, W.: Mendel's Principles of Heredity. 3rd impression, 1913. p. 47. The writer cannot procure HILDEBRAND'S original.

2. LEAKE, H. M.: Studies in Indian Cotton. Journ. of Gen., Vol. I, 1910-1911.

3. GREGORY R. P.: Experiments with *Primula sinensis*. Journ. of Gen., Vol. I, No. 2, March, 1911.

presence or absence of the purple eye in the corolla and the purple color in the leaves.

2. The purple color is due to the presence of the purple cell-sap in the cells of which the purple eye and spot on the leaves are composed.

3. By pedigree culture and cross-testing I, II, IV and V were found to be pure types, while III is not pure, it split into IV and V by self-fertilization.

4. In hybrids of these types the presence of factor or factors of purple color is dominant over the absence of the same, and two reciprocal hybrids between any two pure types behave in the same manner.

5. The F_1 -plant is intermediate in color-intensity between the parents.

6. The eye-color and leaf-color of IV is due to one and the same factor, so that both eye-purples and leaf-purples are associated and simultaneous in appearance, but in type II the purple color appears in the leaf only.

7. The types I and IV give the same results by the double reciprocal crossing as well as by the self-fertilization of any one of these parents i.e. $I \times IV$ or $IV \times I$.

8. Two reciprocals of any hybrids are exactly of the same nature, as far as my materials are concerned.

EXPLANATION OF PLATE XI.

The leaves are represented in nearly natural size, but the flowers (only corollas) are magnified.

- Fig. 1. A leaf and a flower of type II.
Fig. 2. " I.
Fig. 3. " IV.
Fig. 4. " V.
Fig. 5. A leaf and a flower of the hybrid between I and II.
Fig. 6. " I and IV.
Fig. 7. A leaf and a flower of type III.
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1 (II)



2 (I)



3 (IV)



4 (V)



5 (I × II)



6 (I × IV)



7 (III)



On a New Species of Maurolicus, *M. japonicus*.

By

Chiyomatsu Ishikawa.

With Plates XII and XIII.

Dorsal 10 or 11; Anal 8+16; Ventral 7; Lateral

Line 23; Transverse line 7.

The length of the head slightly more than the height of the body and a little less than a quarter of the total length (without the caudal). The length of the snout about one-third of the height of the body, and exceeds that of the interorbital. The eye oval, its vertical diameter a little less than its horizontal diameter which is about one-tenth of the length of the body. The breadth of the iris nearly equal all around; the interorbital space about two-thirds the longitudinal diameter of the eye. The cleft of the mouth nearly vertical, the upper jaw crescent-shaped, its posterior half is thinner and broader than its anterior, and reaches to the middle of the eye. Teeth on premaxillary and dentary; small, pointed and in about three rows. Those of the lower jaw perhaps slightly larger than those of the upper. No palatine and vomerine teeth. The nostrils just between the eye and the end of the snout.

The actual measurements of 12 specimens of *Maurolicus japonicus* from Udu:

Number of Specimens	Total length (without caudal)	Height.	Length of Head.	Snout.	Interorbital space.	Diameter of Eye.
No. 1.	51 mm.	12.0 mm.	13.0 mm.	4.0 mm.	3.0 mm.	5.5 mm.
No. 2.	50 "	10.0 "	13.0 "	4.0 "	3.0 "	5.0 "
No. 3.	48 "	11.0 "	12.0 "	4.0 "	3.0 "	5.0 "
No. 4.	47 "	11.0 "	12.0 "	3.0 "	3.0 "	5.0 "
No. 5.	47 "	11.0 "	10.0 "	3.5 "	2.8 "	5.0 "
No. 6.	47 "	10.5 "	10.5 "	3.5 "	2.8 "	5.0 "

Number of Specimens	Total length (without caudal)	Height.	Length of Head.	Snout.	Interorbital space.	Diameter of Eye.
No. 7.	46 mm.	12.0 mm.	12.0 mm.	4.0 mm.	3.0 mm.	5.5 mm.
No. 8.	46 ..	10.0 ..	10.0 ..	3.5 ..	2.2 ..	4.7 ..
No. 9.	46 ..	11.0 ..	10.0 ..	3.2 ..	2.2 ..	4.8 ..
No. 10.	44 ..	11.0 ..	11.0 ..	3.0 ..	2.7 ..	5.0 ..
No. 11.	44 ..	10.0 ..	11.0 ..	3.0 ..	2.8 ..	4.5 ..
No. 12.	43 ..	11.0 ..	9.3 ..	3.0 ..	2.3 ..	4.5 ..
Total	559 mm.	130.5 mm.	133.8 mm.	41.7 mm.	32.8 mm.	59.5 mm.
Average of 12 specimens	46.6 mm.	10.9 mm.	11.2 mm.	3.5 mm.	2.9 mm.	5.0 mm.

The measurements of the above specimens of *Maurolicus japonicus* from Uodu in percentage of the total length (without the caudal) :

No. of Specimens.	Total length (without caudal)	Height.	Length of Head.	Snout.	Interorbital space.	Diameter of Eye
No. 1.	100 mm.	29.5 mm.	25.5 mm.	7.8 mm.	5.9 mm.	10.8 mm.
No. 2.	100 ..	20.0 ..	26.0 ..	8.0 ..	6.0 ..	10.0 ..
No. 3.	100 ..	22.9 ..	25.0 ..	8.3 ..	6.3 ..	10.4 ..
No. 4.	100 ..	23.4 ..	25.5 ..	6.4 ..	6.4 ..	10.6 ..
No. 5.	100 ..	23.4 ..	21.3 ..	7.4 ..	6.0 ..	10.6 ..
No. 6.	100 ..	22.3 ..	22.3 ..	7.1 ..	6.0 ..	10.6 ..
No. 7.	100 ..	26.1 ..	26.1 ..	8.7 ..	6.5 ..	11.9 ..
No. 8.	100 ..	21.7 ..	21.7 ..	7.6 ..	4.8 ..	10.2 ..
No. 9.	100 ..	23.9 ..	21.7 ..	7.0 ..	1.8 ..	10.4 ..
No. 10.	100 ..	25.0 ..	25.0 ..	6.8 ..	6.1 ..	11.4 ..
No. 11.	100 ..	22.7 ..	25.0 ..	6.8 ..	6.4 ..	10.2 ..
No. 12.	100 ..	25.6 ..	21.6 ..	7.0 ..	5.3 ..	10.5 ..
Average measurements in % of the total length	100 ..	23.9 ..	23.9 ..	7.4 ..	5.9 ..	10.6 ..

The origin of the pectoral fin lies in front of the vertical from the posterior end of the gill-opening, and reaches to about two-thirds the distance between the origins of the pectoral and the ventral. The origin of the dorsal fin, a little behind the base of the ventral, is considerably nearer the root of the tail than to the extremity of the snout; the tip of the ventral fin just reaches the anal, if laid backwards. The origin of the anal in the vertical line below the root of the penultimate ray of the dorsal; the base of the fin nearly twice as long as that of the first dorsal, with its anterior third about double the height of the posterior. The base of the second dorsal nearly as long as that of the first, its origin lies in the

vertical from the middle portion of the anal and ends a little behind the vertical from the root of the last anal ray. The caudal fin moderately forked. The anus lies just in front of the anal fin.

Twelve pairs of luminous organs along the belly from the posterior end of the operculum to the base of the ventral (the thoracico-abdominale), the first pair placed nearer together than the rest; the following pairs nearly of the same size, and arranged in parallel rows; nine in the upper series between the pectorals and the ventrals (the laterale), this series not continued backwards; six pairs between the base of the ventrals and the origin of the anal (the circumventrale), the first two pairs of this series placed along the dorsal base of the ventrals, the third, the fourth, the fifth, and the sixth situated in a curved line beginning with the second and ending with the sixth which is placed at the posterior side of the vent, the concavity of the curve facing ventrally; about sixteen pairs along the base of the anal (the anale), the first pair placed above the others; eight or nine pairs between the anal and the caudal (the pre-caudale), and in the same line with the preceding pairs, the last two placed nearer together; six pairs along the gill-opening (the operculaire) in front of the pectoral fin to the isthmus, the first pair smaller than the others and placed close together; five on the branchiostegal membrane (the branchiostegale); one before (the anti-orbitale), and two below (the suborbitale) the eye; one at the posterior end of the opercle (the post-operculaire); a small pair near the symphysis of the lower jaw.

The numbers of the luminous organs in the thoracico-abdominale, the anale and the precaudale are not constant. Of the thirty-four specimens examined, two possess thirteen pairs of the thoracico-abdominale, one with eleven on the left and twelve on the right; the number differs more with the anale with reference to which among the same thirty-four specimens seventeen pairs are found in nine specimens, in four specimens sixteen on the left and seventeen on the right, two specimens with fifteen on the left and sixteen on the right, one with eighteen pairs, one with eighteen on the left and seventeen on the right, one with eighteen on the left and sixteen on the right, while one with sixteen on the left and fifteen on the right. Lastly, of the precaudale, out of thirty-four speci-

mens examined sixteen specimens with eight pairs, fourteen with nine pairs, one with seven pairs, one with eight on the left and nine on the right, and one with nine on the left and ten on the right.

Numbers of the luminous organs of the thoracico-abdominale, the anale, and the precaudale in the thirty-four specimens of *Maurolicus japonicus* from Uotsu :

Specimens.		Thoracico-abdominale.	Anale.	Precaudale.
No. 1	Left	13	1 + 15	8
	Right	13	1 + 15	8
No. 2.	L.	13	1 + 15	8
	R.	13	1 + 15	8
No. 3.	L.	12	1 + 17	8
	R.	12	1 + 17	8
No. 4.	L.	?	1 + 17	9
	R.	?	1 + 16	9
No. 5.	L.	12	1 + 17	8
	R.	12	1 + 15	8
No. 6.	L.	12	1 + 16	9
	R.	12	1 + 16	9
No. 7.	L.	12	1 + 16	9
	R.	12	1 + 16	9
No. 8.	L.	12	1 + 16	9
	R.	12	1 + 16	9
No. 9.	L.	12	1 + 16	8
	R.	12	1 + 16	8
No. 10.	L.	12	1 + 16	8
	R.	12	1 + 16	8
No. 11.	L.	12	1 + 16	8
	R.	12	1 + 16	8
No. 12.	L.	12	1 + 16	8
	R.	12	1 + 16	8
No. 13.	L.	?	1 + 16	8
	R.	?	1 + 16	8
No. 14.	L.	12	1 + 15	9
	R.	12	1 + 16	9
No. 15.	L.	12	1 + 15	9
	R.	12	1 + 16	9
No. 16.	L.	12	1 + 15	9
	R.	12	1 + 16	9
No. 17.	L.	12	1 + 15	7
	R.	12	1 + 16	7
No. 18.	L.	12	1 + 15	9
	R.	12	1 + 15	9
No. 19.	L.	12	1 + 15	9
	R.	12	1 + 15	9
No. 20.	L.	12	1 + 15	9
	R.	12	1 + 15	9
No. 21	L.	?	1 + 15	9
	R.	?	1 + 15	9

Specimens.		Thoracico-abdominale.	Anale.	Precaudale.
No. 22.	L.	12	1+15	8
	R.	12	1+15	9
No. 23.	L.	?	1+15	9
	R.	?	1+15	9
No. 24.	L.	?	1+15	9
	R.	?	1+15	10
No. 25.	L.	12	1+15	9
	R.	?	?	?
No. 26.	L.	12	1+15	9
	R.	12	1+14	9
No. 27.	L.	12	1+15	8
	R.	12	1+15	8
No. 28.	L.	12	1+15	8
	R.	12	1+15	8
No. 29.	L.	12	1+15	8
	R.	12	1+15	8
No. 30.	L.	12	1+15	8
	R.	12	1+15	8
No. 31.	L.	12	1+15	8
	R.	12	1+15	8
No. 32.	L.	12	1+14	8
	R.	14	1+15	8
No. 33.	L.	12	1+14	8
	R.	12	1+15?	8
No. 34.	L.	11	1+16	9
	R.	12	1+16	9

As to the addition or reduction of the organs in any of the above-mentioned three groups, the thoracico-abdominale, the anale and the precaudale, we can not form any conclusion whether these are restricted to one special side of the animals or not. To state the fact only we observe that, of the thoracico-abdominale, the only one case (No. 34) of this kind, shows that it is the left side one which is reduced in number. Of the anale, out of nine cases, four (Nos. 14, 15 [Fig. 8], 16 [Fig. 6], and 17 [Fig. 9]) with an additional organ on the right, two (Nos. 32 and 33) with one organ less on the left, i.e. fifteen on the left and sixteen on the right; one (No. 4, Fig. 7.) with an additional organ on the right and two additional ones on the left; one (No. 5) with two additional organs on the left; while the other one (No. 26) with an organ less on the right side. It will thus be seen that with the anale, more cases are found with a greater number of organs on the right (six cases) than on the left (three cases). Lastly, with the precaudale, we have one case (No. 22, Fig. 11.)

with eight on the left and nine on the right, and another case (No. 24) with nine on the left and ten on the right (Fig. 12). As to the position of the supernumerary organ in a row, or the reduction of an organ in a row, i.e. whether an organ is interposed between the others, or is added at the ends, or taken away from the ends or somewhere from the row, there appears to be some general rule. In the anale of No. 17, where sixteen organs are counted on the left and seventeen on the right, we have the first pair regularly placed on both sides, and while there are three succeeding organs on the left, there are four on the right, the fourth of the left in the same line with the fifth of the right. By closer examination it will be seen that the third right is smaller than the others, and appears to be the one interposed between the second and the third right, pushing the fourth slightly backwards (Fig. 9). The same is seen with the precaudale of No. 24 where we find, instead of four anterior organs on the left, five on the right (Fig. 12). The case of the anale of No. 14 where the supernumerary organ is seen on the right side, is rather interesting, since here in place of the third anterior organ on the left, there are two small organs on the right, showing thus the probable division of the third one on the right (Fig. 10). To give a case where the reduction appears to have taken place, we take No. 26 (Fig. 5) with sixteen on the left, and fifteen on the right. Here we see the two pairs of organs at the posterior end in regular rows, and closely set together, and while on the left hand side three more organs are added and these also in close set, on the right there are only two, the posterior of which or the third from the most posterior one, is placed a little in advance of the corresponding one on the left, the fourth comes to be placed in a regular row with the fifth of the left. Thus while the five organs on the left side are arranged in close set, the third and the fourth on the right are separated from each other by a space, which appears to show that the fourth one here present is in reality the fifth, the original fourth being dropped off. Similarly with the anale in Nos. 4 (Fig. 7), 16, 32, and 33 and with the precaudale in No. 22, the supernumerary or reduced organs are either added or taken away from the intermediary ones, and not at or from the end, the only one exception being No. 15 where the supernumerary one of the anale on the right hand side,

is due to the addition at the extreme hind end, the last one of the left being parallel with the penultimate one of the right (Fig. 8).

Scales large, thin, cycloid; twenty-three in the mid-lateral line, seven in the transverse line. Four large scales in the mid-lateral line; these are the second, the tenth, the fourteenth, and the seventeenth scales from the anterior end, and gradually decreasing in size from before backwards. Seven pairs of small scales along the ventral side of the gill opening, the six anterior pairs of which lying above the branchiostegal luminous organs; twelve pairs of scales along the ventral side of the body between the posterior end of the last branchiostegal organs and the ventral fins, also lying above the thoraco-abdominal organs; six pairs in front of the vent, lying above the six circum-ventral organs. Many pairs of scales on each side of the anal fin and in front of the caudal, each placed above the luminous organs of these regions. Nine pairs of scales above the thoraco-abdominal organs; and a pair of isolated organs in front of the ventral series of organs, are placed below a pair of scales which form the continuation of the scales placed above the thoraco-abdominal organs.

Gill-rakers long and numerous, twenty-four on the first branchial arch. Pseudobranchiae present, six in number. The blind sac of the stomach rather large, with pyloric appendages well developed, which are nine in number and of variable length; the intestine runs rather straight to the level of the hind end of the blind sac where it makes an s-shaped twisting and runs again straight to the anus (Fig. 13).

The colour of the animal is silvery on side and belly; sepia brown along the dorsal part. Luminous organs on silvery ground with black pigment; photogenic portion milky white. The peritoneum with deep brown pigments.

Habitat: Uodu, Ettyû, Japan Sea; generally caught with a kind of small ground net, the teguri-ami, from the depth of about two to three hundred fathoms, together with other fishes of commercial value. The same fish is also caught in the seas off Idu peninsula on the Pacific side.

General remarks: That this fish is closely allied to the Atlantic species (*Maurolicus pennanti* Walbaum) there is no doubt. It is, however, to be noticed that there are some points of difference between the two

which justify us to consider the present form as a new species. These are the lesser number of the anal fin-rays in our species, than in the Atlantic fish, and consequently the space between the last anal ray and the caudal is longer in the former than in the latter ; thus the eight or nine luminous spots of this portion lying all behind the anal fin in our form, whereas in the Atlantic species only the last two or three spots lie in the same space. Slight differences are also to be observed in the size of the eye in relation to that of the length of the head, and also in the number of luminous organs.

JORDAN, TANAKA and SNYDER in their Catalogue of the Fishes of Japan give a single species of *Maurolicus*, and identify it with the Atlantic form, without giving, however, any definition of the form. Without description of their type specimen, it is not justifiable to consider their fish to be identical with ours. It is very improbable, however, that two nearly allied forms are to be found in one and the same place ; neither was the *pennanti* form found among many specimens observed by the present author, which were taken from the same localities where the specimens of the authors were also taken, namely in the seas of Uotsu and Idu. It is, moreover, improbable that the present form which is one of the most common fishes taken in the bay of Toyama, should have escaped the notice of such an ichthyologist as TANAKA, who personally visited the same place as the present author did, and obtained the specimens. Lastly, in favour of the view of the identity of the present form with that given by the authors, the fact is to be mentioned, that the structure of the luminous organs described by OSHIMA, from the so-called *M. pennanti*, exactly corresponds with that of the present form, even the absence of the reflector in the anteorbital organ being alike in both the forms. All these tend to point to the fact, that the *pennanti* of JORDAN, TANAKA and SNYDER is not the same species as the Atlantic one, but is a species distinct from it.

LITERATURE CITED.

1. GÜNTHER, A., Catalogue of Fishes in the British Museum, Vol. v. p. 387-390.
2. JORDAN, DAVID STARR, and EVERMANN, BARTON WARREN. The Fishes of North and Middle America, 1896, p. 576-577.
3. JORDAN, D. S., S. TANAKA, and J. O. SNYDER. A Catalogue of the Fishes of Japan. Journ. Coll. Sci. Imp. Univ. of Tokyo, 1913, p. 51.
4. OSHIMA, HIROSHI. Some Observations on the Luminous Organs of Fishes, Journ. Coll. Sci. Imp. Univ. Tokyo, 1911, p. 9-15.

EXPLANATION OF PLATES.

PLATE XII.

- Fig. 1. *Maurolicus japonicus*, magnified about 3 diameters.
Fig. 2. The same, ventral view.
Fig. 3. The same, dorsal view.

PLATE XIII.

Figures 4-12 represent the outlines of the luminous organs drawn with Zeiss objective μ^* , compensation ocular 2.

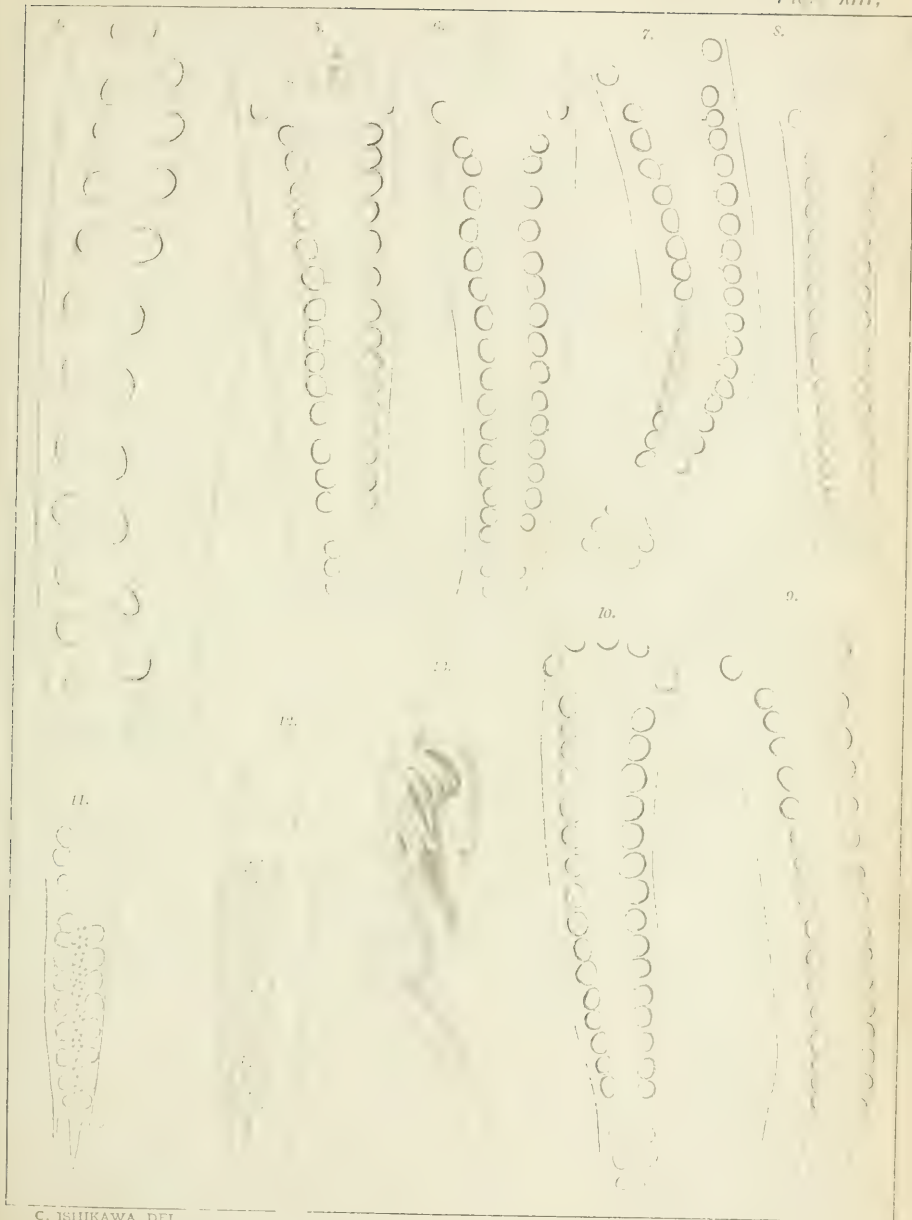
Fig. 4. The thoracic-abdominal of the specimen No. 34, in which a reduction took place on the left side.

Fig. 5-10. The anal of six individuals; Fig. 5 represents those of No. 26; Fig. 6, of No. 16; Fig. 7, of No. 4; Fig. 8, of No. 15; Fig. 9, of No. 17 and Fig. 10, of No. 14.

Fig. 11-12. The precaudal of two individuals; Fig. 11, those of No. 2, and Fig. 12, of No. 24.

Fig. 13. The stomach, the pyloric appendages, the liver and the intestine magnified about 4 diameters.





Notes on *Anguilla mauritiana* Bennett.

By

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In a joint work¹ with Professor CHIYOMATSU ISHIKAWA we stated our opinion about a species of eel known as *kanikui* occurring in the waters of the Bonin islands, and frequently also in the rivers and streams of the middle and southern parts of the main Island of Japan, i.e. that it is distinct from our common eel, *Anguilla japonica*, in contradiction to the statement of such ichthyologists as DAVID STARR JORDAN, JOHN OTTERBEIN SNYDER and SHIGEHO TANAKA, and we have arrived at the conclusion that this eel is synonymous with a species inhabiting the Indo-Malay region and known as *Ang. mauritiana*.² As, however, the specimens at our disposal were then too few, our conclusion might have been looked upon as unsatisfactory, although the points we have considered were clear enough to show the distinctions between the two. Through the kindness of Mr. SIX-YEMON KURUSIMA, we have since obtained sixteen specimens of *kanikui* from the Bonin islands, the examination of which has proved beyond doubt the correctness of our former conclusion, that *kanikui* is identical with *Ang. mauritiana* of southern waters, and distinct from *Ang. japonica*.

Before going further, I wish to express my warmest thanks to Professor ISHIKAWA for his kindness in guiding the present work and in looking over the manuscript. Thanks are also due to Mr. SIXYEMON

1. CHIYOMATSU ISHIKAWA and NISUKE TAKAHASI:—Note on the Eels of Japanese, Formosan, Korean and adjacent Waters. Journ. of the Coll. of Agriculture, Imp. Univ. of Tokyo, Vol. IV, No. 7.

2. It will thus be seen that the stuffed specimen in the Imp. Museum at Ueno, stated to be distinct from *Ang. japonica* by CHIYOMATSU ISHIKAWA, and identified with *Ang. mauritiana* in his "Catalogue of Fishes" is to be taken as being verified.

KURUSIMA who at my request has kindly sent me the specimens.

In the following pages will be given firstly the facts about the measurements of different parts of the bodies either in percentage of or in proportion to the total length, as well as the numbers of vertebrae, pectoral fin rays and of branchiostegal rays, and then an attempt is made to draw conclusions from these measurements.

1. The lengths of the head etc. in percentage of the total length.

The length of the head varies from 10.53 to 17.18; the distance from the gill-opening to the origin of the dorsal fin, from 11.58 to 17.94; the distance between the gill-opening and the vent, from 25.57 to 38.17; the distance between the commencements of the dorsal and anal fins, from 15.23 to 23.28; the length of the cleft of the mouth, from 3.33 to 5.61; the length of the pectoral fin, from 3.50 to 7.02; the length of the snout, from 2.17 to 3.44; the diameter of the eye, from 1.05 to 1.61; the interorbital space, from 2.34 to 3.73; the height of the body in front of the anus, from 5.17 to 7.69 in percentage of the total length.

2. The lengths of the head etc. contained in the total length etc.

The length of the head is contained 5.82 to 9.50 in the total length; 0.78 to 1.14 in the distance of the gill-opening from the origin of the dorsal fin; 1.79 to 2.63 in its distance from the vent; the distance between the commencements of the dorsal and anal fins is contained 0.56 to 0.83; the length of the snout, 4.25 to 5.78; the length of the pectoral fin 2.30 to 3.75, the length of the cleft of the mouth 2.49 to 3.75 in the head; the diameter of the eye is contained 1.50 to 3.00 in the length of the snout, 1.83 to 3.40 in the interorbital space; the height of the body in front of the anus is contained 13.00 to 19.33 the distance from the tip of the snout to the origin of the dorsal fin, 2.85 to 4.38 in the total length.

3. The ratio of the praeanal and postanal parts.

The ratio of the praeanal and postanal parts varies from 1:0.81 to

1:1.62; and the average length of the praeanal part to that of the postanal part is 1:1.36.

4. The average length of the parts of the body in the percentage of the total length.

The length of the head is 13.47; the distance from the gill-opening to the origin of the dorsal fin, 13.53; the distance between the gill-opening and the vent, 28.99; the distance between the beginnings of the dorsal and anal fins, 16.66; the length of the cleft of the mouth, 4.61; the length of the pectoral fin, 4.20; the length of the snout, 2.83; the diameter of the eye, 1.33; the interorbital space, 2.92; the height of the body before the anus, 6.18 in the average of the percentage of the total length.

5. The average length of the head etc. contained in the total length etc.

The length of the head is contained 7.42 in the total length, 1.00 in the distance from the gill-opening to the commencement of the dorsal fin, 2.16 in its distance to the vent; the length of the snout is contained 4.76, the length of the pectoral fin 2.20, the distance between the beginnings of the dorsal and anal fins, 0.81, the length of the cleft of the mouth, 2.92 in the length of the head; the diameter of the eye is contained 2.13 in the length of the snout, 2.22 in the interorbital space; the height of the body in front of the anus is contained 16.18; the distance from the tip of the snout to the origin of the dorsal fin, 3.70 in the total length in average.

6. The number of the branchiostegal rays.

The number of the branchiostegal rays varies from 9 to 11, the mean number being 10.29. Thirteen examples out of twenty-one have 10 rays.

7. The number of the pectoral fin rays.

The number of the pectoral fin rays is counted to vary from 16 to 20, the mean number being 17.59. The number 17 is the most common, being met with in eight specimens out of twenty-one.

8. The number of the vertebrae.

The number of the vertebrae varies from 99 to 107, of which the praecaudal portion counts 40 to 43, whilst the caudal portion, 58 to 65.

The formula of the vertebrae in average is $41.24 + 63.19 = 104.43$.

The accounts above given are taken from twenty-one specimens of this species, and are far too few to draw any conclusion as to the range of the variations of this species, but these when taken together are enough to distinguish the present species from the allied forms. The distinctive characters that are common to all the specimens so far examined and which make the present form different from other species are:—

1. The number of the vertebrae, which varies from 99 to 107, the average being 104.43, while the average number of the vertebrae in *Ang. japonica*¹ is 115.65, in *Ang. vulgaris*² 114.728, in *Ang. bostoniensis* or *rostrata*³ 107.116, and in *Ang. sinensis*⁴(?) of which latter we had the opportunity of studying only a single specimen, the number is 98, thus approaching quite near to one example of *mauritanica* by which the number is counted to be 99.

2. The head is, in the present species, always shorter than the distance between the commencements of the dorsal and anal fins, whereas in *Ang. japonica* it is longer or sometimes a little shorter, in *Ang. vulgaris*⁵ it is equal or somewhat longer, and in *Ang. bostoniensis*⁶ and *Ang. sinensis*(?) it is longer.

3. The length of the head is either slightly more or less than the distance between the origin of the dorsal fin and the gill-opening. In *Ang. japonica* and *Ang. sinensis*(?) it is always distinctly shorter. According to the descriptions of the authors,⁷ the same seems to be also the case with *Ang. vulgaris* and *Ang. bostoniensis*.

4. The extraordinary development of the lips, which are very broad, is not seen in any other allied species.

6. The cleft of the mouth, which in smaller specimens does not

1, 4. CHIYOMATSU ISHIKAWA and NISUKE TAKAHASI:—I.C.

2, 3. JOHNS. SCHMIDT:—Rapports et Procès-Verbaux des Réunions. Vol. XVIII. P. 4-23, 1914.

5, 6, 7. GÜNTHER:—Catalogue of Fishes. Vol. VIII. D. S. JORDAN and B. W. EVERMANN:—American food and game fishes, 1905. EMIL WALTER:—Der Flusssaal, 1910.

extend beyond the posterior edge of the eye, reaches beyond it in larger forms.

This character will thus be observed to vary with the age of the animals. GÜNTHER makes a similar statement from his specimens. Of the above twenty-one specimens of ours three small individuals measuring 131 mm, 174 mm and 235 mm respectively, with the cleft of the mouth not extending beyond the posterior edge of the eye, while in all others, it reaches far beyond the eye.

These characters taken collectively are enough to distinguish the present form from the allied species and to identify it with the species described by GÜNTHER under the name of *Ang. mauritiana* Bennett.

In this connection, it is perhaps worth mentioning a species of eel which in the above cited joint work with Professor ISHIKAWA was doubtfully identified with *Ang. sinensis* MacCelland which besides other points of difference, has a much fewer number of vertebrae than the allied species, namely only ninety-eight. This number is, as will be seen from the table, only one less than that seen in specimen No. 6 of our *Ang. mauritiana*, and makes it more or less approach to the latter species. Since, however, the external characters of this form are different from all other examples of *Ang. mauritiana* thus far observed, it will be proper to leave the question of its identity with *Ang. mauritiana* until more specimens of this form are examined.

1982

No.	contained in the total length etc.							Ratio of the length of the præanal and postanal parts.	Vertebrae.		Number of Branchiostegals.	Number of pectoral fin rays.
	H.	G.O.-D.	G.O.-V.	H.	H.	T.L.	T.L.	S.t.-V.:V.-C.t.	Formula.	Number		
	P.	H.	H.	D.-A.	M.	h.	S.-D.					
1	3.75	1.04	2.22	0.74	3.75	13.10	2.85	1:0.81	41+63	104	10	17
2	3.38	0.95	2.02	0.83	3.67	19.33	4.05	1:1.62	42+63	105	10	19
3	3.50	0.95	2.09	0.82	3.15	16.21	3.82	1:1.41	41+63	104	10	17
4	2.30	1.05	2.23	0.82	3.10	17.43	3.84	1:1.44	41+64	105	9	17
5	3.00	1.17	2.63	0.63	3.16	14.62	4.38	1:1.61	41+64	105	10	19
6	3.56	0.98	2.35	0.70	3.20	16.98	4.02	1:1.37	41+58	99	11	16
7	3.33	0.94	2.36	0.72	3.94	17.65	4.19	1:1.12	40+65	105	10	17
8	3.47	1.10	2.35	0.63	3.35	18.86	3.80	1:1.39	42+64	106	10	17
†9	3.21	0.87	2.00	0.56	3.59	15.93	3.77	1:1.35	41+64	105	11	20
10	3.35	1.13	2.37	0.63	2.74	17.72	3.99	1:1.53	40+65	105	11	16
11	3.41	1.14	2.39	0.58	2.64	16.18	3.65	1:1.31	42+62	104	11	17
†12	2.87	0.91	1.92	0.61	2.75	13.00	3.61	1:1.36	43+64	107	10	16
13	2.81	1.03	2.19	0.61	2.95	17.88	3.87	1:1.47	40+61	104	10	18
14	2.95	0.98	2.16	0.58	2.95	16.07	3.80	1:1.38	42+63	105	10	16
15	3.30	1.02	2.16	0.57	2.49	18.64	3.79	1:1.41	41+64	105	10	18
16	2.15	1.00	2.21	0.61	3.00	15.44	3.74	1:1.33	41+62	103	11	18
†17	4.09	0.99	2.01	0.64	2.83	16.33	3.63	1:1.39	41+63	104	10	17
18	2.24	1.07	2.16	0.61	3.09	16.40	3.49	1:1.29	42+65	107	10	18
19	3.38	1.11	2.22	0.64	2.63	15.61	3.43	1:1.24	41+62	103	10	17
†20	2.72	1.10	2.08	0.64	2.51	15.71	3.38	1:1.31	43+63	106	11	19
†21	2.11	0.78	1.79	0.58	3.03	14.57	3.45	1:1.20	40+62	102	11	20

mouth
the caudal

=gill-opening; D. origin of the dorsal fin; V.=vent; M.=cleft of the
the body in front of the anus; S.t.=tip of the snout; C.f.=tip of

Anguilla mauritiana BENNETT.

[illegible]

T.L.=total length, taken from the tip of the upper jaw to the tip of the caudal fin; H.=length of the head, taken from the tip of the upper jaw to the upper edge of the gill-opening; G.O.=gill-opening; D.=origin of the dorsal fin; V.n.vant; M.=depth of the mouth, taken from the extremity of the upper jaw to the angle of the mouth; P.=length of the pectoral fin; S.=length of the snout; E.=diameter of the eye; I.=interorbital space; h.=height of the body in front of the anus; S.t.=tip of the snout; O.t.=tip of the caudal fin.

† These are reproduced from Table IV of the joint work with Prof. C. Ishikawa, Journ. Coll. Agric. Tokyo Imp. Univ. Vol. IV, No. 7, and are put in their proper places.

On the Homology of the median longitudinal Muscles- Supracarinalis and Infracarinalis- with the Fin-muscles of the dorsal and anal Fins, and their Functions.

By

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(Director Prof. CHIYOMATSU ISHIKAWA)

With Plates XIV-XV and two Text-Figures.

As far as I am aware, two opinions have hitherto been held as to the functions of the supracarinal and the infracarinal muscles of the bony fishes. The one is that of SEITARO GOTO, the other that of CHARLES WILSON GREENE and CARL HASTLEY GREENE.

According to S. GOTO who made his studies on *Carassius auratus*, each of these muscles is divided into two portions: the supracarinalis into that portion which is situated between the occiput and the dorsal fin, which he calls the levator pinnae dorsalis, and into that portion lying between the dorsal and caudal fins, which he calls the dorsal extensor pinnae caudalis. The infracarinalis is also divided into two portions: the levator pinnae analis, which is situated between the pelvic and anal fins, and the ventral extensor pinnae caudalis, which is situated between the anal and caudal fins. According to his view, these parts, as their names show, are considered as the muscles which are concerned with the movement of the median fins, namely, the levator pinnae dorsalis and levator pinnae analis serving to elevate the dorsal and anal fins, and the dorsal and ventral extensor pinnae caudalis to expand the caudal fin.

C. W. GREENE and C. H. GREENE in their studies on the skeletal
[Jour. Coll. Agric., Vol. VI, No. 3, 1917.]

musculature of the king salmon (*Oncorhynchus tshawytscha*) distinguish, like S. GOTO, the supracarinalis into two portions, the anterior which they call the protractor dorsalis and the posterior, the retractor dorsalis. In the infracarinalis, they distinguish besides the parts enumerated by S. GOTO, which they call the protractor analis or retractor ischii, the one lying between the pelvic and anal fins, and the retractor analis that lying between the anal and caudal fins, and a muscle lying in front of the pelvic fin, which they call by the name of protractor ischii. The functions ascribed by the authors to these parts are supposed to be two-fold, i.e. they work firstly as protractor and retractor of the dorsal, anal, and pelvic fins, and secondly they produce strong dorsal and ventral flexions of the body, which latter should be the chief function of these muscles. The names of the part above given are meant to convey the authors' idea as to their functions. It is to be remarked here that the authors appear to have no knowledge of S. GOTO's treatise, which is written in Japanese, in a little book intended for the use of students in their laboratory work, and not as an elaborate scientific investigation. Otherwise the authors, working over the same ground, would have certainly mentioned the difference of opinions in regard to the functions of these muscles.

Observations on various kinds of fishes revealed to me the fact that the median longitudinal muscles mentioned by the above named authors are found in most of them, but they show great variations; that is, in some fishes all the parts of the muscles are present, while in others only a part of them.

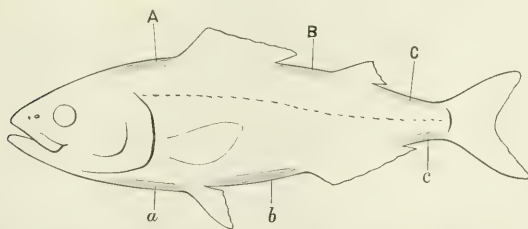
Concerning their functions I have arrived at the conclusion, in contradiction to the statements of S. GOTO, and C. W. and C. H. GREENE, that these muscles do not participate in the movement of the fins. My observations tend to show that they are homologous to the fin-muscles of the dorsal and anal fins, which have become abortive or modified in accordance with the non-development of the fin rays along those portions of the median line where they exist.

As regards the second function stated by C. W. and C. H. GREENE, however, the question still remains whether these muscles are capable of producing the dorsal and ventral flexions of the body, since their idea is based apparently only on the anatomical knowledge of the king salmon, no experiment being recorded.

Experiments on the carp (*Cyprinus carpio*) have shown me that these muscles are not capable of contributing such a motion to the body of the fish. At any rate, these muscles alone cannot produce any such motion in the body of the carp. As, however, I have no knowledge of the king salmon, I do not venture to apply the result obtained by the carp to the king salmon, and cannot positively deny the possibility of these muscles producing such a motion in the fish the authors investigated.

As the specimens thus far observed are too few and as the experiments were carried on only on the carp, my above conclusion may be looked upon as unsatisfactory, but I believe that these facts are enough to answer my question as to the relation between these muscles and the fin-muscles of the dorsal and anal fins, and also to clear up the ideas on the functions of these muscles as stated by S. GOTO and C. W. and C. H. GREENE, which at any rate do not apply to some fishes. For this reason, I venture to state here my view on the functions of these muscles and their relation to the fin-muscles of the dorsal and anal fins.

At the suggestion of Prof. CHIYOMATSU ISHIKAWA, I have tried to use the old terminology of RICHARD OWEN applied to the dorsal and ventral median longitudinal muscles, with the distinctive adjectives: anterior, median or posterior put before the word; thus the supracarinal muscle, that lying between the head and the dorsal fin, will be called the anterior; and that



Text-fig. 1. Diagrammatic figure of a fish, showing the position of supra- and infracarinalis. A, Anterior supracarinalis (Goto's Levator pinnae dorsalis, GREENE's Protractor dorsalis); B, Median supracarinalis; C, Posterior supracarinalis (Goto's Extensor pinnae caudalis, GREENE's Retractor dorsalis); a, Anterior infracarinalis (GREENE's Protractor ischi); b, Median infracarinalis (Goto's Levator pinnae analis, GREENE's Protractor analis or Retractor ischi); c, Posterior infracarinalis (Goto's Extensor pinnae caudalis, GREENE's Retractor analis).

between the dorsal and the caudal fins, the posterior. In a similar way that part of the infracarinalis which lies between the anal and caudal fins will be called the posterior, that lying in front of the pelvic fin, the anterior, while that between the pelvic and anal fins will be the median. When two dorsal fins are present, the part of the supracarinalis which lies between them will be called the median; and when more than two dorsal fins occur, the muscles between them will be called the first, the second etc. median supracarinalis (see Text-fig. 1).

I most heartily thank Prof. CHIYOMATSU ISHIKAWA who lent me the literature and placed the materials at my disposal, together with kind advice in many ways during the progress of the work, especially in the preparation of the manuscript. Thanks are also due to Dr. RINNOSUKE SHŌJI of the Medical College of the Kyoto Imperial University who taught me the methods of physiological experiments.

1. CAN THE SUPRACARINALIS AND THE INFRACARINALIS MOVE THE FINS?

As every one knows, the fin rays of the dorsal and anal fins are jointed to the fin-holders (interneural and interhaemal spines) by free movable articulation. Each fin ray is specially provided with three pairs of fin-muscles—the erector, the depressor and the inclinator, the function of which is to raise or depress; the rays easily without any participation of the movement of the fin-holders. In *Cyprinus carpio* the inclinator muscle of the dorsal fin is poorly developed and the erector muscle of each ray originates partly from the anterior surface of its own holder and partly* from the hind surface of the proximal part of the holder which belongs to the antecedent fin ray, and is inserted on the anterior part of the base of the fin ray; the depressor muscle starts from the hind surface of its own holder and sometimes a small part of it originates from the next and is attached to the posterior part of the base of the fin ray. In addition to such arrangement of the fin-muscles, the fin-holders are firmly fixed in a line along and to the vertebral column by a strong membrane and are deeply inserted between the body muscles; consequently it is not possible to raise or move the fin-holders without breaking them. The caudal fin is

* The erector muscle of the first ray forms naturally an exception on this point.

supported by the caudal extremity of the vertebral column and its movement is performed by the muscles—the plicatores, the flexor ventralis, the flexor dorsalis, the hypochordal, the body or lateral muscles etc.

If we now turn to see how and where the median longitudinal muscles of the fishes, the anterior supracarinalis and the median infracarinalis are inserted, it will be seen that these are inserted only on the first or the most anterior one among many fin-holders of the dorsal and anal fins respectively. The posterior supracarinalis and the posterior infracarinalis are also respectively attached to the hindmost fin-holders of the dorsal and anal fins, and their insertions are placed on the neural and haemal spines of the vertebrae, sometimes in part on the foremost small fin ray of the caudal fin. The anterior infracarinalis is also inserted on the pelvic bone, extending to the isthmus at its anterior end.

The origin and the insertion of these muscles now described leads to the conclusion that they are certainly not capable of raising or moving the dorsal, anal and pelvic fins or expanding the caudal fin.

In order to test the validity of the above conclusion drawn from the anatomical data, experiments were tried in the carp. These were amputation of the muscles, stimulation of the same by the tetanic current from the induction apparatus and by chemical reagents. The muscles were amputated, but showed no sign of disturbance in the movement of the fins and in the act of swimming. Electric currents of varied intensity (0–20 c.m.) were applied to the supra- and infracarinalis but did not cause any sign of movement of the fins. Similar results were obtained by applying to them chemical reagents, such as ammonia, sulphuric acid, nitric acid and hydrochloric acid in strong concentration. Thus the experiments show that the view proposed from the anatomical observations as to the functions of these muscles is valid, disagreeing with the views held by S. GOTO and C. W. and C. H. GREENE. Since these experiments were carried out only on the carp it may be too bold to apply the same conclusion to other fishes, but the similar anatomical feature of their muscles makes it highly probable that they bring forth the same experimental results as obtained from the carp.

2. CAN THE SUPRA- AND INFRACARINALIS PRODUCE THE DORSAL
AND VENTRAL FLEXIONS OF
THE BODY?

C. W. and C. H. GREENE state that the chief function of the supracarinalis and the infracarinalis in the king salmon is to produce the dorsal and ventral flexions of the body.

Observations on the movements of the various bony fishes show us, however, that no such motion* can be recognised on the body while the fish is swimming; and even if such a motion exists, it will be too slight to be seen, and in most cases such a motion may probably be restricted only to the caudal part of the body. The origin and the insertion of these muscles, as stated above, and their rather feeble development in most cases, induce us to believe that these are too weak to produce such an extensive motion as the dorso-ventral flexions of the body. In the region before the caudal part of the body, especially in the fishes whose dorsal or anal fins are situated near the head, the production of such a motion by these muscles lying in front of the fins is not possible, since these are too feeble to produce such a big motion; and even in the caudal region the occurrence of such a motion is very doubtful in most fishes, especially in those in which the finlet is developed, such as *Thunnus*, *Aurisc*, *Euthynnus*, *Cybiu*, *Cololabis*, *Decapterus*, *Katsuwonus*, *Gymnosarda*, *Sarda* and *Acanthocybiu* where the poor development of the posterior carinalis necessarily exclude such a motion. In the carp, in spite of the good development of the carinal muscles, the dorsal and the ventral flexions of the body cannot be produced by them, the electric, chemical and mechanical stimuli on these muscles exercising no effect whatever on the motion of the body and the fins, as stated above. These considerations on the carp may not be applied with equal value to the other fishes, as said before. However, similar conditions prevail in those fishes as in the carp observed by me, so that it will not be quite unreasonable to assume that the conclusion here arrived at can also be applied to them.

* Fishes with elongated bodies such as *Congers*, *Anguilla*, *Misgurnus*, etc. which make serpentine movements naturally form exceptions.

3. CONSIDERATIONS AS TO THE RELATION OF THE SUPRACARINALIS AND THE INFRACARINALIS TO THE FIN-MUSCLES OF THE DORSAL AND ANAL FINS.

The development of the supra- and infracarinalis differs greatly in different kinds of bony fishes. In some, these are well developed, while in others we cannot find even a trace of them. It is a noteworthy fact that in those fishes where the dorsal fin extends to the occiput, the anterior supracarinalis is entirely absent, and where the dorsal or anal fin is continuous with the caudal fin, the posterior supra- or infracarinalis is wanting. These facts, together with the mode of development of these muscles and the innervation from the spinal nerve, make us believe that these muscles are homologous with the fin-muscles of the dorsal and anal fins.

In *Oncorhynchus keta** the "Anlagen" of these muscles take their origin from the myotomes in the same manner as those of the fin-muscles of the dorsal and anal fins, which during the developmental stages take the form of the supracarinalis and the infracarinalis, while those placed at and near the regions where the dorsal and anal fins develop, are transformed into the fin-muscles of these fins. Also, these muscles are innervated by the spinal nerve just as in the fin-muscles of the dorsal and anal fins.

In *Paracentropogon rubripinnis* (Pl. XIV, Fig. 3) and *Limander yokohamæ* in which the dorsal fin extends to the head, the anterior supracarinalis is entirely absent, whereas in *Oncorhynchus keta*, *Scomber japonicus* and *Cyprinus carpio* (Pl. XIV, Fig. 1), where the dorsal fin is situated far behind the head, this muscle is found between the head and the dorsal fin. Now, the absence of this muscle in *Paracentropogon* and *Limander* is to be accounted for by the presence of the fin-muscle of the fin rays. To the same assumption the presence of this muscle in *Oncorhynchus*, *Cyprinus* and *Scomber* is to be looked for, where from the absence of the dorsal fin the fin-muscles became more or less abortive and took the form of the anterior supracarinalis.

Without studying the development of the dorsal fins of *Paracentropogon* and *Limander* this assumption might seem to be rather too bold; but the

* The details will be treated in my next paper now under preparation.

mode of development of this muscle in *Oncorhynchus*, together with the occurrence of the intermediate forms connecting two extremes makes it very probable that this in reality is the case. Thus in *Pagrosomus major* (Pl. XIV, Fig. 2), the anterior supracarinalis is divided into four parts by three spurious interneurals, a fact which tends to show the original paired condition of the muscle, although the fibers of these parts run antero-posteriorly.

In the same way the posterior supracarinalis can be considered as homologous with the fin-muscles of the dorsal fin, the posterior infracarinalis with those of the anal fin. As in the anterior carinal muscle, so we find here also some important facts in favor of this view. Thus, in the fishes in which the finlets are developed, such as *Scomber japonicus* (Pl. XIV, Fig. 4), *Cololabis saira* (Pl. XIV, Fig. 5) and *Auxis maru*, especially in the latter two, the fin-muscles of some finlets do not take the position common to those of the fin rays of the dorsal or anal fins, but show a character which reminds us of that of the posterior carinal muscles, the fin-muscles of the finlet being more or less fused and with their fibres running almost longitudinally, especially the muscles of the hindmost finlet which are in general completely fused together, and form a single muscular bundle. In this last condition it is quite impossible to find any distinction between these and the posterior carinal muscles (see the figure of *Cololabis saira*, Pl. XIV, Fig. 5).

The posterior carinal muscles are always found between the last fin ray of the antecedent fin or last finlet and the caudal fin. Thus in fishes which have only one dorsal fin, such as in *Cyprinus*, the muscle originates from the hindmost interneural of the dorsal fin, in the fishes with two dorsal fins, such as *Mugil cephalus*, it originates from the same of the second dorsal fin; in *Scomber* (Pl. XIV, Fig. 4) and *Cololabis* (Pl. XIV, Fig. 5), which are furnished with finlets, its origin is attached to the holder of the hindmost finlet.

In the same way the posterior infracarinalis always originates from the interhaemal of the last fin ray or from the last finlet, if it occurs. Thus in *Cololabis* and *Scomber* it originates from the hindmost finlet. (Pl. XIV, Fig. 4 and 5).

It is interesting to note in this connection, that when the last fin ray is placed close to the caudal fin, the supra- or infracarinalis is necessarily very short and assumes the shape of the fin-muscles of the ordinary fin rays. This will

be also the case with the muscle or muscles between two or more fins or finlets, as in the case in fishes with continuous fin such as *Anguilla japonica*, *Leptocephalus myriaster* or *Brotula multibarbata* (Pl. XIV, Fig. 6). The comparison of the muscles of *Brotula* with those of *Scomber* and *Cololabis* (Pl. XIV, Fig. 4, 5 and 6) will be of interest, as showing the gradual transition from the ordinary fin-muscles to the posterior supra- and infracarinalis.

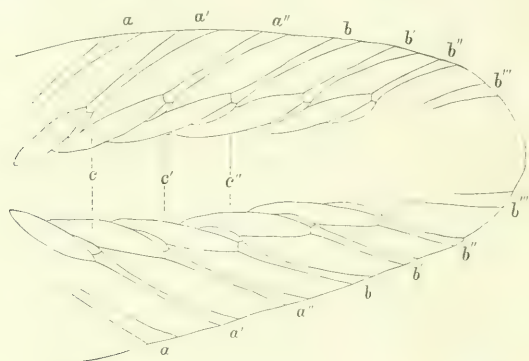
The important contribution to the structure and the phylogeny of the unpaired fins by J. J. SCHMALHAUSEN, is, in this connection, of special interest. To the fin rays of the caudal fin in *Conger* are attached three pairs of muscles, the erectors, the depressors and the incliners, just as to those of the other median fins. A quotation from his statement runs as follows:—

“Hier ist der Ort, auf die Schwanzflosse einiger Teleostier aufmerksam zu machen, bei welchen diese nur physiologisch als solche erscheint, morphologisch aber die hinteren Teile der Dorsalis und Analis vorstellt. Dieses findet z. B. bei den *Apoda* statt; ich habe die Muskultur der Schwanzflosse bei *Conger* untersucht, sie erscheint als unmittelbare Fortsetzung der Muskulatur der Rückenflosse dorsal, und der Afterflosse ventral; an einem jeden Hautstrahle inserieren die für diese Flossen charakteristischen drei Muskelpaare (Musculi inclinatores, erectores und depressores); da die Hautstrahlen der Schwanzflosse durch eine Reihe freier Flossenträger gestützt werden, die von ebensolchen der Dorsalis und Analis nicht unterscheidbar und auch nicht abgegrenzt sind, und ausserdem auf dem äussersten Schwanzende noch ein Rudiment des typischen Teleostierschwanzskelettes, aus drei ganz kleinen Hypuralia bestehend, erhalten ist, so erscheint es unzweifelhaft (es wurde diese Meinung auch früher schon ausgesprochen), dass wir es mit der nach hinten sekundär ausgebreiteten Rücken- und Afterflosse zu tun haben. Bei den *Apoda* ist, wie gesagt, noch ein kleines Rudiment des Skelettes der ursprünglichen homocerkalen Schwanzflosse erhalten geblieben; bei den *Pterasteriidae* ist es aber ganz verschwunden—die primäre Schwanzflosse ist völlig reduziert und durch die Dorsal- und Analflosse funktionell ersetzt worden. Solch eine Schwanzflosse, die aus den zusammengetretenen Dorsal- und Analflossen gebildet ist und sekundär symmetrisch erscheint, hat RYDER gephyrocercal genannt; für solche Flossen (aber nicht für alle sekundär symmetrischen Schwanzflossen, wie man das gewöhnlich tut) soll man diese Bezeichnung beibehalten.

Die Schwanzflosse des *Polypterus* kann also ebenfalls teilweise (nur in ihrer dorsalen Hälfte) als gephyrocercal bezeichnet werden."

Although he does not mention anything about the relation between these muscles and supra- and infracarinalis, his statement is sufficient to support my view on the relation between the fin-muscles of the dorsal or anal fin and the posterior carinal muscles.

The discontinuous fin can be considered as a special case of the continuous



Text-fig. 2. Diagrammatic figure of continuous fin of a fish, showing the relation between the fin-muscles of the dorsal or anal fin and the posterior carinalis.

a, a', a'', b, b', b'', b''', fin rays; c, c', c'', fin-muscles.

fin and we can assume the gradual transition from the continuous to the discontinuous fin and from the ordinary fin-muscles to the posterior carinalis. If the anterior fin rays (Text-fig. 2 *a, a', a''*) are separately inserted from the posterior or caudal fin ray (*b*) and the fin-muscle (*c'*) belonging to the fin ray (*b*) is prolonged, or the fin ray (*a''*) or rays (*a'', b*) have disappeared and the muscles (*c, c'*) of those fin rays are fused, then the continuous fin will be changed into the discontinuous, and at the same time the fin-muscle or muscles (*c'* or *c, c'*) which remained between those newly formed discontinuous fins will take the same relation as in the posterior carinalis and the median fins in the ordinary discontinuous-finned fish.

If we take these assumptions and the above quoted statements of SCHIMMELHAUSEN together into consideration, we shall understand that the

posterior supra- and infracarinalis are homologous with the fin-muscles of the dorsal and anal fins.

4. THE VARIATIONS SEEN IN THE DEVELOPMENT OF THE SUPRA- AND INFRACARINALIS AMONG DIFFERENT FISHES.

If the supra- and infracarinalis are muscles in abortive condition, then it is natural to find great differences in their development, as is usual with all such organs. The following table (Table I) shows that this is the case. It

TABLE I.*

Names of Fishes.	Median longitudinal muscles.					
	Supracarinalis.			Infracarinalis.		
	Anterior	Median	Posterior	Anterior	Median	Posterior
<i>Anguilla japonica</i>	n		n		n	n
<i>Leptocephalus myriaster</i>	n		n		n	n
<i>Enedriæ nebulosus</i>	p		?	n	n	?
<i>Misgurnus angullicaudatus</i>	p		p	n	p	p
<i>Carapus sagamiensis</i>	n		n		n	n
<i>Brotula multibarata</i>	n		n		n	n
<i>Cyclogaster</i> sp.	p		?	?	n	?
<i>Oncorhynchus nerka</i>	p		p	p	p	p
<i>Etmopterus micropus</i>	p		p	p	p	p
<i>Clupea pallasi</i>	p		p	p	p	p
<i>Cyprinus carpio</i>	p		p	p	p	p
<i>Scomber japonicus</i>	p	n	p	n	p	p
<i>Limander yokohamae</i>	n		p	n	p	p
<i>Trichiurus japonicus</i>	p		n		p	n
<i>Paracentropogon rubripinnus</i>	n		p	n	p	p
<i>Pterois lunulata</i>	?		p	n	p	p
<i>Helicolenus emblemarius</i>	p		p	?	p	p
<i>Mugil cephalus</i>	p	p	p	?	p	p
<i>Monacanthus japonicus</i>	n	p	p	n	p	p
<i>Gadus macrocephalus</i>	p	n	n	n	n	n
<i>Cololabis saira</i>	p		p	p	p	p

* p, present; n, absent.

will be seen in the first place that the part belonging to the supracarinalis shows great differences in development. The anterior supracarinalis is entirely wanting in *Paracentrapogon* (Pl. XIV, Fig. 3), *Anguilla*, *Limander*, *Brotula* etc. The first median supracarinalis is well developed in *Mugil* (Pl. XV, Fig. 6. *med. sup.*) and *Monacanthus japonicus** between the first and second dorsal fins, which in *Scomber* is found to be quite obscure, and in *Gadus* (Pl. XV, Fig. 5) no special muscle can be recognised in the same place, which is occupied by the enlargement of the erector muscles belonging to the first ray of the second dorsal fin. The posterior supracarinalis (also infracarinalis) is obscure in *Gadus*.

The median infracarinalis or the muscle lying between the anal and the ventral fins does not always extend from the pelvic bone to the anal fin, and is also very variable in development in different fishes. In *Anguilla*, *Brotula* and *Gadus* this muscle has entirely disappeared; in *Scomber* (Pl. XV, Fig. 7. *med. inf.*), *Cyprinus* (Pl. XV, Fig. 3. *med. inf.*), *Oncorhynchus* and *Etmeneus* it extends from the pelvic bone to the anal fin; in *Paracentropogon* (Pl. XV, Fig. 2. *med. inf.*) it is developed only in the posterior part of the interval between the pelvic and the anal fins. In *Ammodytis personatus* (Pl. XV, Fig. 4. *med. inf.*) where no pelvic fins occur, the muscle is continuous from the anal fin to the isthmus. Thus, while the anterior infracarinalis is quite distinct in *Cyprinus*, *Etmeneus* and *Oncorhynchus*, it is absent in quite a number of other fishes, such as *Anguilla*, *Leptocephalus*, *Enedrias*, *Misgurnus*, *Scomber* etc. In *Oncorhynchus* and *Etmeneus* the anterior infracarinalis is inserted at the anterior end of the pelvic bone, but in *Cyprinus* the posterior end of the muscle is prolonged farther backward, ventral to the muscles of the ventral fin to be inserted in the posterior end of the pelvic bone, immediately before the attachment of the median infracarinalis.

5. ON THE INCLINATOR MUSCLE OF THE DORSAL FIN.

There is an interesting fact as regards the inclinator muscle of the dorsal fin in *Anguilla japonica* (Pl. XIV, Fig. 8. *incl.*), *Scomber japonicus* (Pl. XIV, Fig. 7. *incl.*) and *Clupea pallasi*, which seems to contribute an important

* The first dorsal of this fish is considered by D. S. JORDAN to have been reduced to a stout spine.

confirmation to my conclusion as to the origin of the supracarinalis and the infracarinalis. In most of the fishes the inclinator muscle is found only in the regions of the dorsal and anal fins, being attached on each side of the fin ray, whereas in the above mentioned fishes it is developed even at the places where the fin rays are not found.

In *Scomber* the muscle is attached to the base of the fin ray between the erector and depressor muscles, and with its lower end to the body muscles; in the region of the second dorsal fin its fibres form more or less a compact bundle. In the region of the first dorsal fin and in the space between the first and second dorsal fins, as well as in the median line in front of the first dorsal reaching forward as far as the head, the fibres are rather loosely arranged. Similar arrangement of the inclinator muscle to that on the anterior part of the body in *Scomber* is also seen in *Clupea* and *Anguilla*, in such a way that in *Clupea* the muscle is found along the dorsal fin and forward, while in *Anguilla* it is found along the dorsal line of the fish from right behind the eyes to the end of the tail, and is, moreover, very highly developed.

6. SUMMARY.

From the above observations and considerations I am inclined to conclude that firstly, the supracarinalis and infracarinalis in *Cyprinus* and many other bony fishes can neither move the dorsal, the pelvic, the anal or the caudal fin, and their contraction is, as the experiments on *Cyprinus* show, too weak to cause the dorsal and ventral flexions of the body; and secondly, these muscles were primarily the fin-muscles of the fin rays, which together with the partial elimination of the median fins in most fishes, became rudimentary. This latter fact may possibly contribute a strong argument in favor of CARL RABL's view of the origin of the median fin in contradiction to that of ANTON DOHRN, especially when the view on the homology of the anterior and median infracarinalis with the fin-muscles of the anal fin is proved to be valid.

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EXPLANATION OF PLATES.

List of abbreviations.

<i>A.</i> ,	anal fin.	<i>incl.</i> ,	inclinator muscle.
<i>ant. sup.</i> ,	anterior supracarinalis.	<i>ih.</i> ,	interhaemal spine.
<i>ant. inf.</i> ,	anterior infracarinalis.	<i>med. sup.</i> ,	median supracarinalis.
<i>C.</i> ,	caudal fin.	<i>med. inf.</i> ,	median infracarinalis.
<i>D.</i> ,	dorsal fin.	<i>P.</i> ,	pectoral fin.
<i>dep.</i> ,	depressor muscle.	<i>post. sup.</i> ,	posterior supracarinalis.
<i>I D.</i> ,	first dorsal fin.	<i>post. inf.</i> ,	posterior infracarinalis.
<i>II D.</i> ,	second dorsal fin.	<i>S.</i> ,	spurious interneural spine.
<i>er.</i> ,	erector muscle.	<i>V.</i> ,	ventral fin.
<i>F.</i> ,	finlet.		

PLATE XIV.

Fig. 1. *Cyprinus carpio* L.

Fig. 2. *Pagrosomus major* (T. et S.).

Fig. 3. *Paracentropogon rubripinnis* (T. et S.).

- Fig. 4. *Scomber japonicus* Houttuyn. Side view of the caudal part.
Fig. 5. *Cololabis saira*. Side view of the caudal part.
Fig. 6. *Brotula multibarbata*. Side view of the hind part of the continuous median fin.
Fig. 7. *Scomber japonicus*. Dorsal view.
Fig. 8. *Anguilla japonicus* T. et S. Dorsal view.

PLATE XV.

- Fig. 1. *Scomber japonicus*. Ventral view.
Fig. 2. *Paracentropogon rubripinnis*. Ventral view.
Fig. 3. *Cyprinus carpio*. Ventral view.
Fig. 4. *Ammodyltis personatus* Girard. Ventral view.
Fig. 5. *Gadus macrocephalus* (Tilisius). The parts of the first and second dorsal fins. Side view.
Fig. 6. *Mugil cephalus* L.. The first and second dorsal fins in side view.
Fig. 7. *Cyprinus carpio*. Cross section of the place just behind the occiput.
Fig. 8. *Cyprinus carpio*. Cross section far behind Fig. 7.
Fig. 9. *Cyprinus carpio*. Cross section at the part behind the dorsal fin.
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Ant. inf. 1. Ant. II

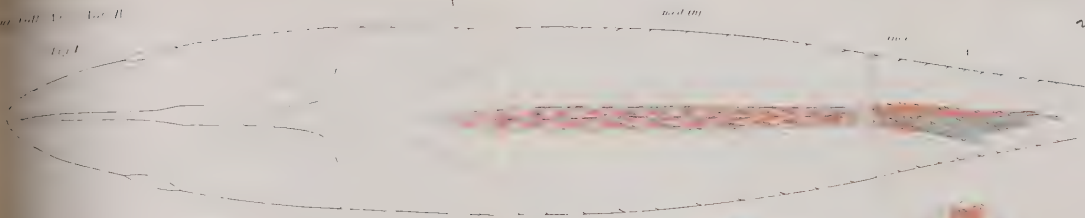


Fig. 2

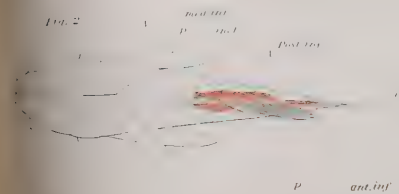


Fig. 3



Fig. 4

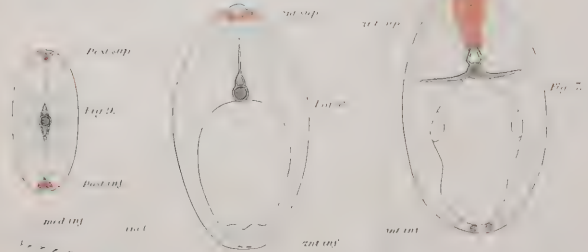
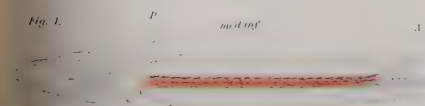
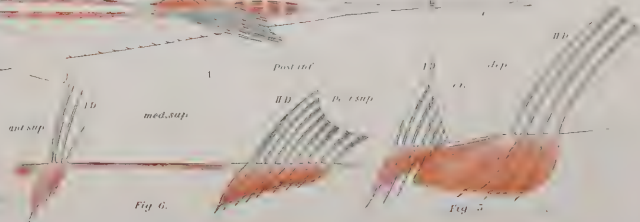


Fig. 6



The Spermatogenesis of an Orthopteron, *Atractomorpha Bedeli* Boliv.

By

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(From the Laboratory for Agricultural Zoology,
Director, Prof. CHIYOMATSU ISHIKAWA.)

With Plate XVI-XVIII.

The present study was undertaken with the object of following in detail the spermatogenesis of an Orthopteron, *Atractomorpha bedeli* Boliv. The spermatogenesis of this Orthopteron gives evidence of the presence of an unpaired modified chromosome or so-called accessory chromosome which shows peculiar behavior, especially in its staining capacity, in the meiotic and in the post-meiotic generations, and also in the phase immediately preceding the meiotic. The author is much indebted to Prof. ISHIKAWA, for suggesting to him this subject for study and for the aid and facilities given him during the progress of this work, especially for the working out of the manuscript. Thanks are also due to Prof. YATSU of the Science College who kindly lent the necessary literature on the subject.

I. Materials and Methods.

Atractomorpha bedeli is a green or occasionally brown colored small short-horned grasshopper (Acrididae) which has a conical shaped head and is commonly found in the fields near Tokyo. The size of the male and the female differ very much, the former being about one inch long and the latter one inch and a half. The testes are paired and lie in the abdominal cavity, surrounding the alimentary canal. The testicular follicles are white in color and connected one with the other by yellowish fibers. Each follicle is club-shaped,

with its stalk opening into the *vás deferens*. The number of the follicles in each testis is about twenty to twenty-five, rarely twenty-eight, being variable with different individuals. Spermatogenetic stages of every description may be found in the testes of any individual, but for the observation of young spermatogonia larvae are more desirable. As a rule, the cysts situated further away from the blind end of the follicle represent more advanced stages than those placed near it, but these may not always be found in order of development. All the cells in a single cyst are generally in about the same stage.

The materials used in these studies were obtained in the garden of the College of Agriculture at Komaba. The testes which were taken out from the abdominal cavity were placed in the normal salt solution, and by means of fine needles the follicles were then separated from each other and immediately transferred into fixing agents. For the fixation FLEMING's chromo-aceto-osmic acid mixture (weak formula) and an aqueous solution of sublimate were used, but this latter was almost useless for observations.

Several stains were tried. Of these HEIDENHAIN's iron-haematoxylin gave the best result, but FLEMING's tricolor stain and DELAFIELD's haematoxylin were frequently found to be of use for control. Sections were cut five or ten micra thick.

As the various stages could be seen in a single section, and the order of the development was conveniently traced out, longitudinal sections were more favorable for observation than transverse ones; but as to the views of the spindles there was no great difference in the direction of the sections, since these lie in every possible direction in a cyst.

Smearcd preparations were also used with advantage, as in the sections the spiremes were liable to be cut into pieces, or some chromosomes of a single cell might come to be placed in different sections which made the number of chromosomes uncertain. The smearing was done with needles with which the follicles were opened and the contents quickly spread on the slide. The object was now exposed to the fumes of 1% solution of osmic acid for two to three minutes, next immersed into 70% alcohol and stained with the same stains as used for the sections. The preparations thus made gave no good results, the cells swelled and the chromosomes changed their forms more or less, but they were found to be useful for the control of the

sections. The entire shape of the mature spermatozoon was best studied by teasing them out of the follicles which were preserved as mentioned above, stained with DELAFIELD's haematoxylin and macerated with 2% chloral hydrate.

II. Observations.

A. THE SPERMATOGONIA.

Young spermatogonial cells are not packed together as is seen in older ones. They are situated at the blind end of the follicle, and attached to the wall. The nucleus is relatively large as compared with the amount of the cytoplasm, and the chromatin are scarcely visible (Fig. 2).

The nucleoli have irregular shapes and their size varies. In many cases their number is found to be four but sometimes three, and more rarely two or one. These variations in the number of nucleoli are either caused by the coalescence of the four primary ones, or by the miscalculation of the number produced from the scattering of the nucleoli in the process of sectioning. That the former explanation appears to be true is seen from the fact that where there are three nucleoli present in a nucleus, one is considerably larger than the other two. The nucleoli give the appearance of granular construction composed of aggregated chromatin granules which remain undiffused, while the other chromatin granules lose their staining capacity. They are to be observed only in the resting cell, but become later concealed among the spiremes. The behavior of the nucleoli in the cell of the ovarian follicles and oogonia is quite similar to that of the spermatogonia, becoming also concealed among the spiremes in later stages of development (Figs. 71, 73).

At the commencement of the prophase the chromatin granules become gradually apparent, presenting a reticular appearance. They arrange themselves in rows, which are probably situated on the linins, but never become diffused irregularly throughout the nucleus. The reticular condition of the threads can be traced to some distance, even in the earlier stages (Figs. 3, 4).

The spiremes thus formed represent a peculiar appearance and look like contorted elastic threads which are distributed throughout the whole space of the nucleus (Fig. 5). The spiremes become gradually denser and thicker until

the contorted character is lost. At the stage in which the spiremes have lost their contorted character, the longitudinal splits are clearly visible (Figs. 6, 7). In a dense spireme stage it is impossible to observe whether the spiremes are continuous or not, owing to the great contortion. As, however, in a stage immediately following this, the individual segments can be made out, it seems very probable that they are segmented even in the earlier stages. The position of the segments, which are at first irregular, gradually come to take the form of a radial rosette which probably represents the equatorial plate of the ensuing stage (Fig. 8). The spiremes continue to become shorter and thicker until they assume short rod shapes (Fig. 9). The nuclear membrane is now dissolved, and the spindle is formed (Fig. 10).

In the polar view of the equatorial plate of the metaphase, the number of the chromosomes may be counted as nineteen which are arranged radially around an open central space where one or two chromosomes are observed. Departures from this characteristic number are observed in the cells of the same testis. It is, however, impossible to say, owing to the difficulty of getting a correct count, whether these departures really exist. But the possibility of the existence of the variation in number may become more probable after the observation of the metaphase of the first spermatocytes.

The chromosomes vary regularly in size from the largest to the smallest. As many authors have pointed out, it is very probable that they occur in pairs with an exception of a single unpaired one. But unfortunately the differences in size between the neighboring chromosomes in the series are too little and, moreover, the length and thickness of the individual chromosomes differ so much that it is difficult to recognize exactly the paired ones, and the unpaired one is also difficult to be made out. Though in the lateral view of the metaphase the chromosomes appear very crowded, the individual chromosomes can be traced by carefully focussing the microscope.

Every chromosome divides longitudinally along the split which has appeared previously. The division commences at one extremity of the chromosome, drawing the daughter chromosomes toward each pole, thus showing that the insertion of the spindle fibers is at the end of the chromosomes and not median (Figs. 10, 15, 16).

When the halves of most of the chromosomes thus separated have reached

or are about to reach the poles of the spindles some chromosomes are seen with their ends behind the others owing to the greater length. SUTTON ('00), OTTE ('07) and McCLUNG ('08) have described one of such chromosomes as the accessory chromosome, not only on account of its greater length but because of its behavior. In *Atractomorpha* more than one chromosome lags behind the others in the division, a fact which makes it difficult to ascertain which one of these is in reality the accessory chromosome. This, however, becomes apparent in the telophase of the secondary spermatogonia where one chromosome can distinctly be recognized among the others by its behavior.

At the commencement of the telophase the chromosomes are aggregated and soon they regain more or less rosette arrangement, retaining their individualities, and become less compact and granular. Each chromosome indicates the longitudinal split which seems to be the preparation of division for the next mitotic cycle (Fig. 17). After they remain in this condition for a while, they diffuse gradually throughout the nucleus. In this condition, it is difficult to determine whether the cell is at an early prophase or at the later telophase (Figs. 3, 4, 18).

In the resting stages of the younger spermatogonia, the chromosomes are diffused completely, but in those of the later spermatogenic generations the diffusion of the chromosomes becomes incomplete and the nuclei pass over quickly to the prophase of the next mitotic cycle. As the generations advance the prophase becomes also shorter and therefore contorted spiremies as that shown in Fig. 5 are scarcely to be observed.

In the last spermatogonia, spaces are produced between the cells, due to the increasing of the volume of the cyst before they enter the metaphase (Fig. 1 d). But in certain cases which happen usually in larval or young testes, the spaces are produced after the cells end the spermatogonial mitosis and enter the growth period (Fig. 1 e).

In the telophase of the secondary spermatogonia the behavior of the chromosomal disintegration is different in some respects from that in the primary spermatogonia. Here all the chromosomes do not become diffused as in the primary spermatogonia, but one of them does not disintegrate and remains almost homogeneous in structure but with a slight loss of staining capacity. This undiffused chromosome may be considered as an accessory chromosome as

judged from the behavior hereafter to be described. It migrates from the rosette and comes to lie close to the nuclear membrane where it lies until the cell enters the metaphase of the first spermatocytes (Figs. 19-23).

Comparison of the ovarian follicle cells and oogonial cells with the primary spermatogonia above described, shows that the processes are almost homologous but the secondary spermatogonia represent some diversities in this, that in the female cells the undiffused or accessory chromosome is never to be found within the nuclei, although the nucleoli are visible as in the spermatogonia (Figs. 70-77).

B. THE SPERMATOCYTES.

a. The Growth Period.

As the telophase of the spermatogonia passes gradually to the growth period, the distinction between the two can scarcely be traced. But for the sake of convenience the stage in which the chromatins appear to reach final diffusion will be taken as such (Fig. 25).

The earliest stage of the growth period, as already described, can easily be distinguished from the younger spermatogonia by the perpetuation of the accessory chromosome. The cells increase in size, becoming three or four times as large as they were at the beginning of this stage and the spaces produced between the neighbouring cells in the metaphase or the telophase of the last spermatogonial division become completely obliterated (Figs. 26, 27). The growth of the cells is accompanied by that of the nuclei, so that the relative size remains constant, the nuclei being always comparatively large. Throughout the growth period the nucleoli are distinguishable as an apparently aggregated mass of chromatin granules which are deeply stained. They often approach the accessory chromosome and unite with it or they unite with one another (Figs. 25, 26). These unions, however, appear to be only the result of attachment, and not the conjugation, phenomena similar to this being observed in other cells at resting stage; where no conjugation is expected to happen. When the spiremes appear and the chromatins regain the staining capacity the nucleoli can not be distinctly seen (Figs. 27, 28).

b. The Ordinary Chromosomes in the First Spermatocytes.

Even in the stage when the ordinary chromosomes are entirely diffused,

the chromatin granules do not distribute irregularly within the nucleus and seem to be so arranged along the linin fibers that they form a network of delicate granulated threads. Together with the growth of the cell the granulated threads gradually increase their staining capacity and thickness (Fig. 26), and at last when the cells fill up the space of the cyst, these appear as convolute threads uniformly scattered within the nucleus (Fig. 27).

Even in very delicate threads, the granules appear to be arranged in double rows, thus showing evidence of longitudinal splits in the spiremes. The synizesis stage can not be recognized, a fact which has often been described by investigators of the spermatogenesis of Orthoptera. The double character of the spiremes becomes more evident as the stage advances, until it can be clearly recognized as the longitudinal split. This thickening and shortening of the spiremes together with the increasing of the staining capacity continues and the spiremes represent a coarse appearance, containing deeply staining chromatin granules (Figs. 28-30).

Though the spiremes, apparently, seem to be very convoluted at the stage shown in Figs. 29, 30, careful observation shows that they consist of some loops which are aggregated. The spiremes are, however, too convoluted to show their exact number at these stages, but it is very probable that they are of constant number, which coincides with the number of the ordinary chromosomes in the metaphase. Moreover, the relative size of the spiremes may differ as much as the chromosomes. In the earliest stage or in the growth period it is not possible to say whether the spiremes are continuous or not, owing to their bad staining and to their very delicate structure (Fig. 26). But that the spiremes are not continuous at little later stages is evident from the fact that they clearly represent many blind ends, even though individual ones can not be traced.

The spiremes still continue to shorten and homogeneous bodies are formed instead of granular ones. At the same time, these become compact and show a tendency to aggregate at the central portion of the nucleus (Fig. 31). It is, however, impossible to assert whether in every case in this stage the chromosomes assume this tendency, as the chromosomes, in certain cases, appear as if passing over directly to the ensuing stage, as shown in Figs. 32, 33. But it is certain that the aggregation of the chromosomes is not

produced artificially, since the cells in a single cyst are found in this condition while the cells in the neighboring cyst show the ensuing stage.

The chromosomes now change their form and represent the characteristic appearance found usually in the metaphase of the first spermatocytes of Orthoptera (Figs. 32, 33). Longitudinal splits which become once indistinct in those stages where the chromosomes aggregate at the central portion of the nucleus, become again clearly visible. The transverse cleft becomes also visible at the point where the paired spermatogonial chromosomes conjugated, thus showing the preparation of the reducing division. The nuclear membrane disappears and both the longitudinal and the transverse clefts become again scarcely visible when this last stage is attained.

In the metaphase, beside one accessory chromosome, nine ordinary chromosomes are found which represent half the number found in the spermatogonia (Figs. 34-36). But in some cells, though rarely, eight ordinary chromosomes are observed, an accessory chromosome being always present (Figs. 37-39). As this last number can be exactly counted not only in sections but in smeared preparations where the possibility of miscounting may be out of question, we can conclude that there is a variety as to the number of chromosomes in the spermatocytes of *Atractomorpha*. An observation of the metaphase shows the various forms and sizes of the chromosomes. Tendency to construct the various forms is recognizable from the stage when the spiremes begin to be compact structures. Since the length, the thickness and probably also the form of corresponding chromosomes in different cells differ more in this stage than in a spermatogonial metaphase, it is very difficult to recognize the corresponding chromosomes in the different cells, as in *Melanoplus bivittatus* (NOWLIN, '08), *Syrphula admirabilis* (ROBERTSON, '08) or *Locusta viridissima* (OTTE, '07). But the smallest chromosome is always recognizable, not only by its smallest size but also by its rod or dumb-bell shape which makes it convenient for the comparison of size. The largest one is, however, often indistinguishable on account of its varying form.

The final construction of the chromosomes in the metaphase shows them to be rod, dumb-bell, cross, ring, and more or less modified shapes. These latter are in the shape of a bent rod, or a cross in which both or one arm is more or less bent so as to make the entire chromosome appear as

asymmetrical, or the cross axis of the chromosome assumes the form of a complete or incomplete ring. Of all these forms, the dumb-bells are always assumed by smaller chromosomes.

Observations of the late prophase just preceding the metaphase show that these various shapes may be referred to a simple rod type, formed by conjugation of paired chromosomes of the spermatogonia. The arms of the cross are formed by the bulging out, on both sides of the conjugated ends, of the chromosomes at right angle to the original rod which now becomes the axis of the cross. The only difference between the dumb-bell and the rod lies in the degree of the attachment of the component chromosomes after they have conjugated. Either the conjugated chromosomes may retain their original round forms, or their united ends become more or less flattened and thus they pass gradually to the simple rod. The ring is formed by union of the free ends of the bivalent chromosome. In the late prophase, there are many other shapes, such as V, Y, K, 8 etc., and these are to be interpreted as forms assumed by the bivalent chromosomes in their formation of rod, cross or ring.

Details as to the discussion of the tetrad formation above given have been already described by many investigators of the spermatogenesis, such as PAULMIER ('99), MONTGOMERY ('00, '11), McCLUNG ('02), GROSS ('04, '06), OTTE ('07), ROBERTSON ('08), WILKE ('07), BUCHNER ('09) and McCLUNG and PINNEY ('11). In this respect *Atractomorpha* forms no great deviation except in a few points which will be discussed later in the general consideration.

The nuclear membrane now disappears and the ordinary chromosomes take their position in the equatorial plate of the spindle with their longitudinal axes parallel to the axis of the spindle, except the rings. These are so placed that the plane of the ring lies perpendicular to the axis of the spindle, thus, in the lateral view, making it appear like a rod laid parallel to the equatorial plate (Fig. 40).

Every chromosome regains its original rod shape when it is just about to divide. The restoration is produced by drawing in of the arms of the cross toward the axis, or by the separation of the united end of the ring above described (Fig. 40).

The line of conjugation of the component chromosomes becomes more and more conspicuous, and the chromosome separates into its components at

this line (Fig. 41). From this behavior it is evident that in this mitosis the chromosomes are divided transversely and therefore it is a reduction-division. Further discussion on this point will be given in the general consideration.

When the chromosomes thus separated travel toward the respective pole, the posterior end of each flies apart and a V or U shape is produced (Figs. 41, 42). Migration of the chromosomes toward the poles is so rapidly carried out that the stage shown in Fig. 42 is rarely found in the sections.

As soon as the chromosomes arrive at the poles they so closely aggregate that the individual chromosomes are scarcely distinguishable (Fig. 44). But soon after this the staining capacity of the chromosomes becomes less and their individuality can again be made out. Each now shows somewhat granular structure (Figs. 45, 46). From the observation of this stage and of that shown in Fig. 43 which represents a stage just before that of Fig. 44, it is most probable that the last condition of the chromosomes of the first spermatocyte is the V or U shapes assumed during the anaphase, and thus their individuality is most probably maintained.

c. The Ordinary Chromosomes in the Second Spermatocytes.

The faintly stained chromosomes above described become again more and more distinct by increasing their staining capacity, their outline becomes smooth, and the homogeneous chromosomes are thus formed (Figs. 47-49).

Here it should be noticed that at the late telophase of the first spermatocytes the chromosomes become massed together and cause the nucleus to appear smaller than it really is, and as a result of this, this phase is often apt to be mistaken for the spermatids. But from the fact, that in this phase the size of the nucleus itself remains constant as a whole, while the space occupied by the chromosomes decreases, and that this phase is often found in the cyst in which many of the cells are in the metaphase of the second spermatocytes (Fig. 49), it is evident that these cells are in fact the prophase of the second spermatocytes, and not the spermatids, the cells of which are in fact much smaller than these (Figs. 56, 57).

The prophase of the second spermatocytes is short, and when the chromosomes have assumed homogeneous condition the nuclear membrane disappears. In the metaphase the chromosomes quickly take the radial arrangement with their longitudinal axes placed on the equatorial plate of the spindle, contrary

to the metaphase of the first spermatocytes. The number of chromosomes is generally nine in every cell besides one accessory chromosome wherever it occurs (Figs. 49, 50).

Most of the chromosomes assume a V-shape with a narrow angle, the apices of which are as usual directed toward the axis of the spindle. The two arms of the V are generally contained on the plane perpendicular to the equatorial plate of the spindle (Figs. 51, 52). Neither ring nor cross is found. It is clear that the two limbs of the V are nothing else than chromatids separated by the longitudinal split, because the form, size and thickness of the limbs are very like those assumed in the anaphase of the first spermatocytes as already mentioned. It can easily be considered that the opening of the split which has occurred in the preceding mitotic cycle, may be repeated in the metaphase or in the anaphase of this stage. The behavior of the accessory chromosome in this stage gives more positive proof that the angle of the V is a longitudinal split of the chromosome. In this way, the chromosomes are divided longitudinally and the second spermatocyte mitosis is consequently an equational-division. The chromosomes in the anaphase are in the shape of a more or less bent rod but never in any other shape (Figs. 53, 54).

As in the first spermatocytes, when the chromosomes have reached the poles they are so closely massed together within the nucleus that the individual chromosomes are difficult to distinguish (Fig. 55). At the same time, they lose gradually their staining capacity before they begin to diffuse (Fig. 56).

After the diffusion of the chromosomes there remain, besides some minute granules, some deeply stained bodies of various sizes and forms, some of which are sometimes confluent with the accessory chromosome (Figs. 57, 58). Their number seems to be constant, coinciding with those in the spermatogonia. It is easily conceivable that these bodies may correspond to the nucleoli which have appeared in the previous resting stages, as already described.

d. The Accessory Chromosome.

It has already been mentioned that one chromosome does not diffuse in the telophase of the secondary spermatogonia, while the others become almost diffused (Figs. 21, 22). In the growth period it regains its homogeneous construction in contradistinction to the others in which the diffusion proceeds further, so that it becomes much more conspicuous in this than in preceding

stages. This chromosome is the accessory chromosome, or more suitably the chromosome-nucleolus of WILSON.

At the stage passing from the telophase of the last secondary spermatogonia to the growth period, the accessory chromosome changes its position and becomes closely attached to the nuclear membrane. At the same time its shape becomes discoidal, the outer or convex surface of the disc facing the nuclear membrane (Figs. 26-28). In this condition it remains through the growth period and early prophase of the first spermatocytes. With the growth of the nucleus, its size also increases until it becomes as large as that seen in the metaphase. On careful observation, the longitudinal split of the accessory chromosome is visible already in the secondary spermatogonia when it can be recognized as a chromosome-nucleolus.

As the spiremes regain the staining capacity the chromosome-nucleolus shows a tendency to assume the characteristic form of a chromosome (Figs. 29, 30). It becomes rod shape and detaches from the nuclear membrane. In the late prophase when the ordinary chromosomes have assumed smooth contour and compact structure, no positively distinguishable difference can be observed between the accessory and the ordinary chromosomes (Figs. 31-33). From the later behavior, however, it is very probable that the accessory chromosome occupies the position at the periphery of the chromosome group.

In the metaphase, when the ordinary chromosomes assume their final condition as already described, the accessory chromosome represents such a peculiar condition, especially in staining capacity, that it can be definitely recognized as such. Contrary to the ordinary chromosomes which have the tendency of increasing the staining capacity in these stages, the accessory chromosome shows less staining capacity for HEIDENHAIN's iron-haematoxylin than in the prophase, only a small portion at both extremities of the rod, especially the end facing the pole of the spindle, being deeply stained (Figs. 34, 37-41). With other staining methods the differentiation is not so clear as with iron-haematoxylin (Figs. 35, 36). During this stage it is always rod-shape, no other shapes being seen. The longitudinal split seen in earlier stages is also clearly recognizable, but no trace of a transverse cleft or constriction is seen as in the ordinary chromosomes. Such a peculiar staining capacity of the accessory chromosome as seen in *Atractomorpha* is, as far as the author knows, very

rare. GROSS ('06) found that in the tetrad formation stage or in an earlier stage of the first spermatocytes of *Pyrrohocoris apterus*, the accessory chromosome represents a more faintly stained appearance than the other chromosomes with iron-haematoxylin, but this differentiation becomes mostly lost when the spindle is formed. In the second spermatocytes it was also sometimes stained lighter with iron-haematoxylin. Of the second spermatocytes of *Oedipoda* BUCHNER ('09) also gave a short description as follows: "Bei einer DELAFIELD-Hämatoxylinfärbung ist das accessorische Chromosom meist etwas blasser gefärbt als die übrigen Chromosomen." Besides, according to the observation of STEVENS ('10), the smaller heterochromosomes in the second spermatocytes of *Forficula auricularia*, which had been divided precociously "were paler blue than the other chromosomes, a difference which is often noticeable between the larger (x_1) and smaller (x_2) heterochromosomes in the metaphase of the first maturation mitosis in preparations stained with thionin," the same being noticed for the lagging chromosome of the mitosis of the same stage.

The accessory chromosome is generally found near one pole of the spindle, while the other chromosomes are still in the equatorial plate. It is evident from the observation of the metaphase and from that of the ensuing anaphase, that the accessory chromosome does not divide but travels bodily to one pole of the spindle, producing two series of the second spermatocytes, one of which possess the accessory chromosome and the others not. In the anaphase the longitudinal split which was previously visible as a faint line, becomes conspicuous, owing to the separation of the chromatids. The separation of the chromatids sometimes occurs only along the median portion, both ends remaining in contact, and thus an elongated ring-like figure is produced but sometimes the posterior end of it springs apart in V-shape as in the case of the ordinary chromosomes (Figs. 41, 42). In the advanced stage of the anaphase, the accessory chromosome is caught up by the ordinary chromosomes in spite of starting ahead of the latter (Fig. 42).

At the late anaphase or the commencement of the telophase when the ordinary chromosomes are so aggregated that the individual chromosomes become indistinguishable, the accessory chromosome is also concealed among them (Figs. 43, 44). But soon after, when the ordinary chromosomes represent granular condition, the accessory chromosome becomes gradually distinguishable on account

of its peculiar behavior. Like in the telophase of the secondary spermatogonia it regains the staining capacity and remains in homogeneous condition, and assumes no granular condition all through this phase contrary to the ordinary chromosomes (Figs. 45, 46).

Its form is that of a bent rod with a faint longitudinal split which corresponds to that in the prophase or the metaphase of the first spermatocytes (Figs. 46, 47). It is, however, found not to lie so closely attached to the nuclear membrane as in the case of the first spermatocytes.

At about the time when it enters the metaphase of the second spermatocytes the accessory chromosome loses some staining capacity except at both ends, and resumes a similar condition, but not so clearly differentiated as in the first spermatocytes. The longitudinal split which was very faintly visible in the preceding stage widens a little and a very narrow V-shaped chromosome or a two-parallel rod-figure is produced (Fig. 50). In the equatorial plate, it assumes a similar position to the others with its longitudinal axis parallel to that plane. In the lateral view of the spindle the component chromatids appear to lie immediately one above the other, as the ordinary chromosomes (Fig. 51).

The accessory chromosome divides along the split and its components travel toward the respective pole as a simple rod, in company with the ordinary chromosomes (Fig. 53). Two series of spermatids are thus produced, the one from the second spermatocytes in which the accessory chromosome is present, and the others from those in which it is absent. When all the chromosomes have arrived at the pole, the accessory chromosome becomes indistinguishable from the others, owing to their massed condition, so that, two series of the spermatids are, at this stage, not distinguishable from each other (Fig. 54). But, later on, as the chromosomes lose the staining capacity, the accessory chromosome becomes visible as a deeply stained rod-like body (Figs. 55, 56).

c. The Idiozome and the Mitochondria.

At the commencement of the growth period when the chromosomes of the secondary spermatogonia are diffused or are diffusing, an irregular, faintly stained cytoplasmic body can be seen by careful observation. It always lies close to the nuclear membrane but it seems to have no relation to the spiremes as in that of *Euschistus* described by MONTGOMERY ('11). Its outline can not be distinctly made out (Figs. 22, 23). As the stage advances this body becomes

clearer and maintains a position where a great amount of cytoplasm is found, and thus the polarity of the cell body results. (Figs. 25, 26) In the smeared preparations it stains more deeply than in sections and appears granular (Fig. 24). This body may be the idiozome,* but no centrosome can be found within it; and therefore it is to be recognized that, in this case, the idiozome has no connection with the centrosome.

Besides the body described as the idiozome, there appear one or more smaller bodies which lie either close to or separated from the nuclear membrane (Figs. 27, 29, 30). These bodies are sometimes stained a little more deeply than the idiozome. They may correspond to the mitochondria†.

The mitochondria become gradually indistinct as they increase in size, owing to the loss of the staining capacity. At the advanced stages when the chromosomes assume homogeneous condition, the idiozome and the mitochondria become indistinguishable from one another (Fig. 31-33). Moreover, at the commencement of the metaphase only one such structure is visible at one side of the cell. But it is impossible to say whether the mito-

* MONTGOMERY ('11) proposed the term "sphere" for the cytoplasmic body which has no special relation with the centrosome. This term cannot be applied, however, to this body on account of its behavior which will be described later on. It is also not an "archoplasm" or a "mitosome," since no relation is to be observed between this body and the spindle fibers either in the prophase or in the telophase.

† The bodies described by different authors under the term "mitochondria" are, at the beginning of their appearance, quite variable, but in the end these give rise or contribute to the formation of the "Nebenkern." Thus in *Euschistus* (MONTGOMERY, '11) the mitochondria represent the thread-like structures scattered throughout the cell body. In *Locusta viridissima* (ORTE, '07) the mitochondria which lie around the nucleus as fine granules in the spermatogonia, condense to form a compact "Mitochondrienkörper," within which rings composed of granules appear, and which soon break down into the rings and are distributed into daughter cells. The mitochondria of *Hydroneta lacustris* (WILKE, '07) are small sticks with thick ends, which surround the nucleus and form a conspicuous broad band, the "Mitochondrienkörper." In the spermatocytes of *Forficula auricularia* (ZWEIGER, '06) the crescent-like "Mitochondrienkörper" is composed of threads which have bead-like structures. The mitochondria of the *Oedipoda* (BUCHNER, '03), which lie in the cell as irregular lumps with fine granules, become lodged, especially, on the peripheral layer of the spindle fibers in the mitosis of the first spermatocytes. The bodies described as "yolk granules" by PAULMER ('99) for *Anasa tristis*, and "Dotterkügelchen" or "Dotterkügeln" by HENNING ('91) and GROSS ('06) for *Pyrrhocoris apterus*, may correspond to the mitochondria. Beside these bodies, GROSS observed in two individuals the bodies which he termed "Pseudo-chromosomes," which may be more identical with the mitochondria. It is probable that the structure described by WILCOX ('96) for *Caloptenus femur-rubrum*, where he says: "there are stainable particles on the fibers, and some of the fibers are thickened for a part of their length," though it was found in only one individual, is caused by mitochondria which look very like those of *Atractomorpha*.

chondria persist and the idiozome disappears as in *Euschistus* (MONTGOMERY '11). From the fact, however, that the idiozome is observed at a little earlier stage, and is distinguished from the mitochondria, it may be recognized that the idiozome persists through all the stages and lies in company with the mitochondria. The peculiar crescent shaped structure which lies along the side of the spindle in the metaphase and also along the side of the connecting fibers in the anaphase and the telophase, seems to be composed of the idiozome and the mitochondria (Figs. 40-42). When the cell becomes constricted and the cell plate appears this structure is also crossed by the cell plate in the middle portion (Fig. 43); and becomes divided into two portions to be carried on into the respective cells. Thus by the time the nuclear membrane appears this structure gives rise to a Nebenkern (Figs. 45-47). It is impossible to state by mere observation whether the connecting fibers take part in the composition of the Nebenkern or not.

The formation of the Nebenkern of the spermatids takes place almost similarly to that of the second spermatocytes. It is more distinctly visible than that of the second spermatocyte on account of being more deeply stained (Figs. 48, 49, 51-57).

f. The Centrosomes.

In the metaphase and anaphase of the spermatogonial and the spermatocyte mitosis, the centrosomes are observed as exceedingly minute bodies at the poles of the spindle (Figs. 10, 40, 41, 51, 52). They are too minute to be recognized in the cells of other stages even though they may be present. It can, however, be positively stated that they are never found within the idiozome. *Atractomorpha*, at any rate, is not a favorable material for the study of the behavior of the centrosomes.

C. THE SPERMATIDS UP TO THE FORMATION OF THE SPERMATOOA.

In the spermatids above described, the ordinary chromosomes disintegrate into minute granules and are scattered throughout the nucleus. Some chromatin masses, however, remain undiffused, and so does the accessory chromosome, if present (Figs. 57, 58). These chromatin masses undoubtedly correspond to those in the growth period and are, therefore, the chromatin-nucleoli of WILSON. They are recognized during the transformation of the spermatids into the spermatoo-

zoa where they become concealed within the heads which assume a homogeneous condition.

The accessory chromosome or chromosome-nucleolus retains its original rod-form for some time, occupying any position in the nucleus. After a little while it changes into an irregular mass, and finally assumes a somewhat round form in which it remains. The result of the division of the first spermatocytes should produce an equal number of spermatids with both the chromosome-nucleolus and some chromatin-nucleoli and those without the former but the latter only. This seems really the case from actual observation, though the accurate counting of the cell is not always possible. In the stage in which the chromosomes are almost entirely diffused, the centrosome is not recognizable (Fig. 58). This becomes, however, in a short time observable as a minute particle which lies closely attached to the nuclear membrane near the Nebenkern, at the insertion of the axial filament to the head (Figs. 59, 60). Afterwards two slightly larger centrosomes are clearly seen which lie side by side (Fig. 61). It is impossible to say with certainty whether they have been divided into two at this position or were already divided. But the observation of the section as shown in Figs. 59, 60 shows the latter to be most probable, because a minute particle, which is united by the fine filament with the centrosome, is seen to be also a centrosome.

During these changes, the cell-membrane gradually loses its distinctness and the outline of the individual cell becomes indistinguishable (Figs. 61, 62). Accordingly the changes occurring in the cytoplasm during the development of the spermatozoon and the final position of the cytoplasm in the ripe spermatozoon can not be stated.

At first, the axial filament runs alongside the Nebenkern but does not pass through the latter. Later on, however, the Nebenkern surrounds the axial filament and commences to elongate to form the sheath of the tail and assumes a spindle shape (Fig. 61). Owing to the great length of the tail it is very difficult to trace it to the distal portion of one and the same spermatozoon.

When the development of the tail is completed, the anterior portion of the nucleus elongates and forms a pointed apex, while the posterior portion remains still roundish (Figs. 63, 64).

In the mean time, the chromatin-nucleoli and the chromosome-nucleolus, if present, lose their staining capacity and the head assumes homogeneous structure with the deeply stained centrosomes at the insertion of the tail. But there are some heads which are stained uniformly and deeply, contrary to most found in the same cyst, a fact which seems to be ascribable to the action of the reagent. The head gradually elongates and when it reaches the final elongation, it becomes very long like a thread, (Figs. 65-69). At last all the heads become stained deeply and homogeneously, and the centrosomes are therefore concealed from view.

During these stages, the body called "mitosome" or "aerosome" which finally forms the "lance" or "Spitzenstück" of the mature spermatozoon is not visible. The sharp point at the anterior end of the spermatozoon seems to be formed by the elongation of the nucleus itself as above described.

At the time when the tails are formed, the position of the spermatids within a cyst is changed gradually in such a manner that their anterior ends are turned toward the blind end of the follicle, and the tails lie parallel with their free ends projecting toward the lumen of the cyst.

III. General Considerations.

A. ON THE LONGITUDINAL SPLIT.

GROSS ('04, '06) and ROBERTSON ('08) have described the first spermatocyte mitosis as equational, though the chromosomes apparently enter the spindle with the longitudinal split perpendicular to the equatorial plate of the spindle, the tetrad having the ability to change its axis from a longitudinal to a transverse direction. NOWLIN ('08) has also come to the same conclusion and stated that "the chromosome during the prophase begins to separate into identical halves along the longitudinal split and practically completes the movement by the time it enters the spindle." The description of the process given by GROSS ('06) is as follows: "Ich nehme an, dass auch bei *Pyrrhocoris* die Ausbiegung der Berührungsenden zweier conjugierter und längsgespaltener Chromosomen nicht wieder rückgebildet wird, sondern fortschreitet bis zum Austausch der Spalthälften, so dass jede Dyade am Schluss des Vorgangs aus 2 ungleichnamigen Längshälften besteht und die Tetrade den Bau $\frac{ab}{ab}$ hat." In *Atractomorpha*,

however, the cross (which is formed of original rod never shows a moving out of the longitudinal axis in transverse direction but remains in the same direction. The chromosomes which always remain in dumb-bell or rod shape have no way of changing the direction of the axes. When such chromosomes which have no ability to change the direction of the axes, are placed with their longitudinal axes parallel to that of the spindle and are divided transversely, this mitosis should be reduction. This is also the case with the ring which, as before stated, is produced by the union of the free ends of the rod.

It is now necessary to discuss whether the chromosomes which appear in the first spermatocytes are those produced originally by the parallel conjugation, known as parasynesis, and the longitudinal split appearing along the individual spireme is the sign of the separation of the two original chromosomes thus united, or the chromosomes conjugate end to end from the first, and the longitudinal cleft is the true longitudinal split. Both OTTE ('07) and MORSE ('09) have described the bivalent chromosomes as formed by the parallel conjugation, but while the former author considered the ensuing two spermatocyte mitoses as transverse but not longitudinal division, the latter said that "each of the spermatocyte mitoses involves a longitudinal division of the chromosomes, etc."

In *Atractomorpha*, as already described, the chromatin granules in the spiremes seem to be arranged in two rows even in the final diffusion stage, that is, early growth period, and as the development advances, this double character becomes more clear, but no behavior of the parallel conjugation can be recognized. On the other hand, it is more reasonable to consider the longitudinal cleft as a true longitudinal split when it is traced to the spermatogonia, where it can no longer be doubted that it is not on account of the parallel conjugation of the two chromosomes, because the number of the chromosomes would be otherwise too many as compared with that in the spermatocytes, being as many as four times to the latter. If the chromosomes retain their individuality, as many investigators believe, the splits which appear in the prophase of the spermatogonia are to be considered as the reappearance of the same which are produced in the telophase of the preceding mitotic cycle. It may be apprehensible that in the same way the longitudinal split which appears after the last spermatogonial division may persist through the growth period and reappear in the prophase of the next mitotic cycle, that is, in the prophase

of the first spermatocytes, even if it were concealed entirely from view during the diffused condition; the more so, as the arrangement in two rows of the chromatin granules can be traced, though not very clearly.

B. ON THE ACCESSORY CHROMOSOME.

In *Atractomorpha*, as already described, some deeply stained bodies always appear in the resting stage of the spermatogonia, while most of the chromatin granules almost lose the staining capacity. These bodies may not, however, correspond to WILSON's chromosome-nucleolus which gives rise to the accessory chromosome, but to his chromatin-nucleoli which "designate any compact deeply staining chromatin-mass, present in the resting nucleus, which afterward contributes to the formation of the chromosomes." It is certain that they neither give rise to such small chromosomes as the "microchromosome" of WILSON in such form as *Alydus* or *Anasa* etc., nor to the "minute chromosomes" of MONTGOMERY in *Euschistus*, since such minute bodies corresponding to these are never found in the metaphase of *Atractomorpha*. Neither do they appear to be allied to the "supernumerary chromosomes" described by STEVEN ('08) for some species of *Diabrotica*, which behave as heterochromosomes, though in the spermatogenesis of *Atractomorpha* some variations in the number of the chromosomes are observed.

It is very difficult to prove the presence of the accessory chromosomes by counting the number of the chromosomes not only on account of the difficulty in getting a correct count but the variations as to the number of the chromosomes. The difficulty of exactly counting the chromosomes has been pertinently expressed by FOOT and STROBELL ('07) in the following words: "we realize in common with all cytologists the difficulty of getting a correct count of so large a number of small bodies crowded into a contracted space. If two or more chromosomes are in such close contact that their line of separation is obscured a correct count is impossible." Moreover, in the spermatogonia of *Atractomorpha*, more or less than nineteen chromosomes would often be counted which makes it difficult to determine whether these are in even or odd numbers, and consequently to recognize the presence of the accessory chromosome by the count only. It is, therefore, necessary to recognize it by the observation of its behavior.

The study of the spermatogenesis of insects reveals the presence of equally or unequally paired heterochromosomes as well as an unpaired one in Hemiptera, Coleoptera, Diptera and Euplexoptera, though there are some controversies between the investigators on some species. In Orthoptera the heterochromosome, on the contrary, appears generally as unpaired, that is, univalent in the spermatocytes, with a few exceptions. MONTGOMERY ('05) described the paired heterochromosomes in *Syrbula acuticornis* which ROBERTSON ('08) in his study on *Syrbula admirabilis* maintained to be a mistake, and said that "his (MONTGOMERY'S) mistake is due, in part at least, to some large chromatin-like nucleolar structures that are present, at first as two bodies and later as one, in the resting period between the last spermatogonial and the first spermatocyte division." SCHELLENBERG ('13) observed, in *Diestrammena marmorata*, a bivalent heterochromosome, the components of which are separate in the young spermatogonia, while later on they conjugate into V- or U-shape in which it persists throughout the ensuing stages, and the following explanation was given: "Was nun bei *Diestrammena* vor allem für die Ableitung des accessorischen Chromosoms von einem ursprünglich paarigen, bzw. von zwei Chromosomen spricht, ist nicht sein Verhalten in der Spermatocyte, sondern das häufige Vorhandensein der zwei stabförmigen Teilstücke in den jungen Spermatogonien mit allen Übergängen zur einheitlichen Chromatinschleife, die sich in den letzten Spermatogoniengenerationen schliesslich allein vorfindet, eine Erscheinung, die mit der These, dass das Heterochromosom mit der Generationshöhe der männlichen Keimzelle immer stärker zutage tritt, sehr gut übereinstimmt."

In *Atractomorpha*, the nucleolas-like bodies which appear in the resting stages, as above described, are quite different from the accessory chromosome or partner of the paired heterochromosomes but correspond to WILSON'S chromatin-nucleoli. The accessory chromosome shows no evidence of its bivalent nature throughout all the stages. In later spermatogonial generations when it first comes into recognition, it appears as a large chromosome but not the largest of all. A longitudinal cleft seen in the accessory chromosome of this stage might be looked upon as a parallel conjugation of two univalent components, but it is to be considered that this is not the case from the observation of the ordinary chromosomes, each of which also shows the similar longitudinal cleft, which, as stated above, is no doubt due to the longitudinal

split. In the spermatocytes, it shows also no aspect of its being bivalent, excepting the longitudinal cleft which is the continuation of the same split observed in the spermatogonia. Moreover, it is not proper to think that the heterochromosomes alone conjugate parasyndetically while the ordinary chromosomes do so metasyndetically.

IV. Summary.

1. At the spermatogonial prophase the spiremcs look like contorted elastic threads which seem not to be continuous. Soon afterwards, these are arranged in radial rosettes and become shorter and thicker to construct the rod-shaped chromosomes of various sizes, probably in pairs. The longitudinal split in each spireme is visible from the first.

2. In the telophase, the chromosomes do not diffuse immediately, but become granular in structure and represent longitudinal clefts.

3. Complete diffusion of the chromosomes may be restricted in the young spermatogonia and as the spermatogenic generations advance they pass over quickly to the prophase of the next mitotic cycle. In the last spermatogonia the cells become more or less separated from each other.

4. In the resting stages the chromatin granules never seem to be irregularly distributed through the nucleus but arranged along the linin fibers. In the first spermatocytes, they are observed as doubled, which is the continuation of the last spermatogonial mitosis.

5. There is no synösis stage, but in the late prophase the chromosomes represent the tendency to aggregate at the central portion of the nucleus in some cells, but not in all. The spiremcs seem not to be continuous.

6. In the metaphase of the first spermatocytes the chromosomes represent various forms, such as, rod, dumb-bell, cross, ring, etc. and different sizes. The number of the chromosomes is generally counted as 19 in spermatogonia and 10 in the first spermatocytes including the accessory chromosome which has no mate to conjugate. But departures from these numbers can be observed.

7. In the metaphase of the first spermatocytes, the chromosomes are arranged in the equatorial plate with their longitudinal axes, parallel with the axis of the spindle, except the rings, which lie with their diameters coinciding

with the equatorial plate. The first spermatocyte mitosis is transverse or reduction.

8. During the migration of the chromosomes toward the respective pole, the chromosomes open the proximal ends and assume the V- or U-shape.

9. The prophase of the second spermatocytes is brief, and the chromosomes appear as short and thick spiremes, which are at first granular but soon become homogeneous. In the metaphase the chromosomes are arranged with their longitudinal axes coinciding with the equatorial plate of the spindle. Most of the chromosomes assume narrowly opened V's. The second spermatocyte mitosis is longitudinal or equational.

10. Some deeply stained nucleoli always appear in every resting stage of the spermatogenesis after the chromosomes have diffused. These bodies seem not to give rise independently to the accessory chromosome or any other chromosome. Female cells also contain these bodies.

11. Presence of the accessory chromosome can be distinctly recognized in the telophase of the secondary spermatogonia. The accessory chromosome does not diffuse hereafter until it is concealed in the head of the mature spermatozoon which shows homogeneous structure.

12. Throughout the growth period and the prophase of the first spermatocytes, the accessory chromosome occupies its position attached closely to the nuclear membrane. When the ordinary chromosomes assume homogeneous condition the accessory chromosome represents no distinguishable difference. In the metaphase, however, it becomes less stained and always assumes rod-shape with longitudinal split but never shows transverse cleft or tetrad-form. It does not divide in the first spermatocyte mitosis but passes over to one of the daughter cells and consequently it is not contained in one half of the series of the second spermatocytes.

14. In the metaphase of the second spermatocytes, the accessory chromosome again loses its staining capacity, and divides longitudinally along the split. It becomes concealed for a time among the mass of the chromosomes, appearing again after the ordinary chromosomes are diffused.

15. The idiozome appears as a cytoplasmic body in the growth period. It seems to have no connection with the centrosome. Mitochondria appear as one or more small bodies in the early prophase of the first spermatocytes.

They increase in size and become indistinguishable from the idiozome. A crescent-shaped structure which lies along the spindle in both the first and second spermatocytes seems to be composed of the idiozome and the mitochondria. This structure gives rise to a Nebenkern.

16. In spermatogonia and in spermatocytes the centrosomes are so minute that they can not be distinguished from other particles except when they are found at the poles of the spindle. In the immature spermatozoon, two centrosomes can be observed which are placed side by side on the nuclear membrane at the insertion of the axial filament.

17. The Nebenkern which surrounds the axial filament elongates to form the sheath of the tail. After the tail has developed completely, the nucleus elongates so slenderly that it is almost indistinguishable from the tail. A sharp anterior point of the spermatozoon seems to be formed by the elongation of the nucleus itself and not by other particular structures.

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EXPLANATION OF FIGURES.

All the figures were drawn by the author with the aid of Abbe's Zeichenapparat at the level of the microscope stage, with the magnification afforded by a Zeiss apochromatic immersion

objective, 2 mm. and a compensating ocular 6 (tube length 175 mm.), except Figs. 12-14 which were drawn with a compensating ocular 8 and Figs. 35-39 with a compensating ocular 6, (tube length 145 mm.), and Fig. 1 which was drawn from a far smaller magnification.

PLATE XVI.

Fig. 1. Entire view of a longitudinal section of a testicular follicle showing the position of spermatogenetic stages. *a*, resting stage of the primary spermatogonia; *b*, prophase of the primary spermatogonia in which spiremes begin to appear; *c*, telophase of the primary spermatogonia; *d*, metaphase and anaphase of the last secondary spermatogonia; *e*, beginning of the growth period; *f*, growth period; *g*, first spermatocyte prophase; *h*, late first spermatocyte prophase; *i*, second spermatocyte metaphase and anaphase; *j*, spermatids; *k*, immature spermatozoa heads which have not yet reached to final elongation; *l*, mature spermatozoa.

Fig. 2. Primary spermatogonia; resting stage, found at the blind end of a follicle. Four nucleoli are to be observed.

Fig. 3. Beginning of prophase of the primary spermatogonia. Nucleoli are still recognizable.

Fig. 4. Stage following Fig. 3, nucleoli very difficult to be seen.

Fig. 5. Prophase of the primary spermatogonia. The spiremes present a contorted thread-like appearance.

Figs. 6, 7. Later stage than the above. The spiremes lose their contorted character and present the longitudinal split.

Figs. 8, 9. Late prophase of the primary spermatogonia. Spiremes or chromosomes assume their position in radial rosette and are becoming shorter and thicker. The chromosomes of the cells are not all represented, some being transferred to the other sections.

Fig. 10. Polar and lateral view of metaphase of the primary spermatogonia. Former shows nineteen chromosomes. Chromosomes in one of the cells begin to separate.

Fig. 11. Polar view of the same stage as Fig. 10. The left cell shows more than nineteen chromosomes; in the right one, some chromosomes were transferred into another section.

Fig. 12. Polar view of metaphase of the last secondary spermatogonia. Number of the chromosomes are counted to be nineteen.

Figs. 13, 14. The same. Number of the chromosomes are counted variously as seventeen, eighteen or nineteen, some of the chromosomes being in close contact.

Fig. 15. Anaphase of the primary spermatogonia.

Fig. 16. The same stage of the last secondary spermatogonia. Some of the chromosomes remain with their ends behind the others.

Fig. 17. Telophase of the primary spermatogonia. Chromosomes are arranged in radial rosette and assume granular condition, representing longitudinal split.

Fig. 18. Stage following Fig. 17. Chromosomes almost diffused, accessory chromosome indistinguishable.

Figs. 19-23. Successive stages of telophase of the last secondary spermatogonia. Chromo-

somes after assuming an arrangement of radial rosette diffuse gradually. The accessory chromosome, however, remains undiffused and migrates to attach to the nuclear membrane. In Figs. 22, 23, idiozome is recognized as a faintly stained cytoplasmic body.

Fig. 24. The same stage as above, drawn from a smeared preparation, which shows the idiozome clearly.

Fig. 25. Beginning of the growth period. Nucleoli can be recognized.

Fig. 26. Early prophase of the first spermatocytes. The space between the cells still a little to be seen.

Figs. 27-30. Prophase of the first spermatocytes, showing successive stages in which the spiremes become gradually distinct on account of their increasing straining capacity and of shortening. Longitudinal split clearly observable in every spireme. In addition to the idiozome, mitochondria appear as smaller bodies, stained somewhat deeply.

Fig. 31. Stage in which the chromosomes aggregate at the central portion of the nucleus, representing tendency to form the various shapes. The accessory chromosome becomes indistinguishable from the others. Mitochondria is scarcely distinguishable from the idiozome.

Figs. 32, 33. Stage following the above. Various shapes of the chromosomes are observable.

Fig. 34. Metaphase of the first spermatocytes. Ten chromosomes are present in both cells, all visible in one section. The accessory chromosome can be recognized as a faintly stained body at the periphery of the group.

Fig. 35. The same stage as Fig. 34, drawn from smeared preparations stained with DELA-FIELD's haematoxylin. The accessory chromosome cannot be distinguished.

PLATE XVII.

Fig. 36. The same as Fig. 35.

Figs. 37-39. The same as above, drawn from smeared preparations stained with iron-haematoxylin. These cells contain, as an exceptional case, only nine chromosomes together with an accessory.

Fig. 40. Lateral view of the metaphase of the first spermatocytes, the middle cell being in oblique view. The accessory chromosome lies near one pole. Some ordinary chromosomes are restored to the original rod shape. The fact that the rings are arranged coinciding with the equatorial plate of the spindle may be recognized by comparison with the oblique view. Mitochondria can be seen as a crescent shaped body at one side of the spindle.

Fig. 41. Beginning of the anaphase of the first spermatocytes, showing various conditions of the separation of the chromosomes. Central large chromosomes in the upper cell are going to return to the original rod shape from the cross. Ordinary chromosomes just separated in the lower cell assume V-shape and the longitudinal split of the accessory chromosome separates at the middle portion.

Fig. 42. Stages following Fig. 41. Mitochondria come to be placed between the daughter chromosome groups.

Fig. 43. Late anaphase of the first spermatocytes. The accessory chromosome may often be distinguished on account of its lesser staining capacity. Cell plate appears.

Fig. 44. Very late anaphase. The accessory chromosome indistinguishable.

Fig. 45. First spermatocyte telophase. The accessory chromosome again recognizable in one daughter cell as deeply stained homogeneous body. Longitudinal splits are visible in all chromosomes.

Fig. 46. Very late telophase. All chromosomes mass together in the nucleus so as not to be distinguishable from one another, except the accessory chromosome.

Fig. 47. Beginning of the second spermatocyte prophase. Chromosome-nucleolus is absent in one cell. Nebenkern present.

Fig. 48. Stage following Fig. 47, showing successive changes in the formation of the spiremes.

Fig. 49. Early prophase and metaphase of the second spermatocytes, lying adjoined in one cyst. Latter shows nine ordinary chromosomes but no accessory; former represents chromosome-nucleolus.

Fig. 50. Polar view of the metaphase of the second spermatocytes, left cell containing nine ordinary chromosomes and an accessory, the right one with nine ordinary chromosomes but no accessory.

Fig. 51. Lateral view of the metaphase of the second spermatocytes, both cells containing the accessory chromosome.

Fig. 52. Lateral view of the metaphase and anaphase of the second spermatocytes. No accessory chromosome to be recognized.

Fig. 53. Stage following Fig. 52. The accessory chromosome separates longitudinally and migrates with ordinary chromosomes.

Fig. 54. Late anaphase of the second spermatocytes. The accessory chromosome becomes indistinguishable. Mitochondria lie between two daughter chromosome groups.

Fig. 55. Telophase of the second spermatocytes. Chromosomes mass together.

PLATE XVIII.

Fig. 56. A further stage. Ordinary chromosomes lose their staining capacity. The accessory chromosome becomes again recognizable, owing to its staining capacity. Mitochondria begin to condense to form the Nebenkern.

Fig. 57. Early spermatids, the chromosome-nucleolus being absent in two of them. Ordinary chromosomes disappear, leaving only some nucleoli.

Figs. 58, 59. Spermatids. The nucleus increases its size a little. Chromatin granules again appear. The chromosome-nucleolus begins to change its form.

Fig. 60. A little later stage than above. Nebenkern of the lower cell surrounds the axial filament and elongates more or less.

Fig. 61. Stage following above; two centrosomes increasing in size laid side by side. Cell membrane becomes indistinct. Nebenkern elongates to spindle shape.

Fig. 62. Stage following Fig. 61. Nebenkern elongates more and more. Chromosome-nucleolus assumes round shape.

Figs. 63, 64. Nucleus begins to elongate at the anterior portion to form a pointed apex. Chromosome-nucleolus loses its staining capacity and becomes scarcely visible.

Figs. 65-68. Successive elongation of the head.

Fig. 69. Head of the mature spermatozoon.

Fig. 70. Terminal chamber of the ovary.

Fig. 71. Beginning of the growth period of the oocytes, nucleoli being visible.

Fig. 72. Nucleus of the growth period following Fig. 71.

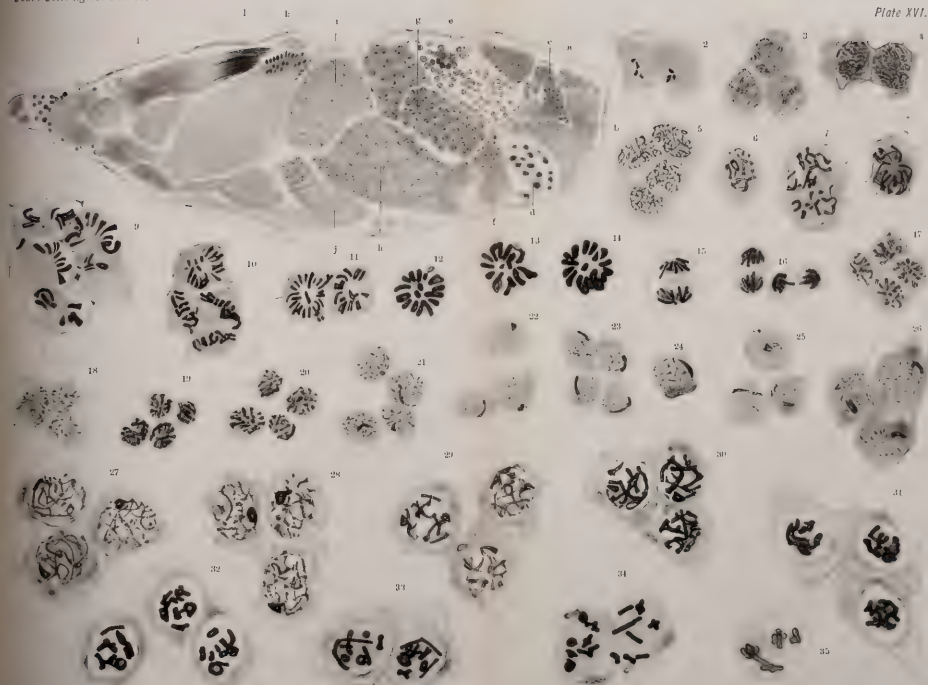
Fig. 73. Resting stage and prophase of the ovarian follicle cells which correspond to Fig. 2 and Fig. 3 respectively.

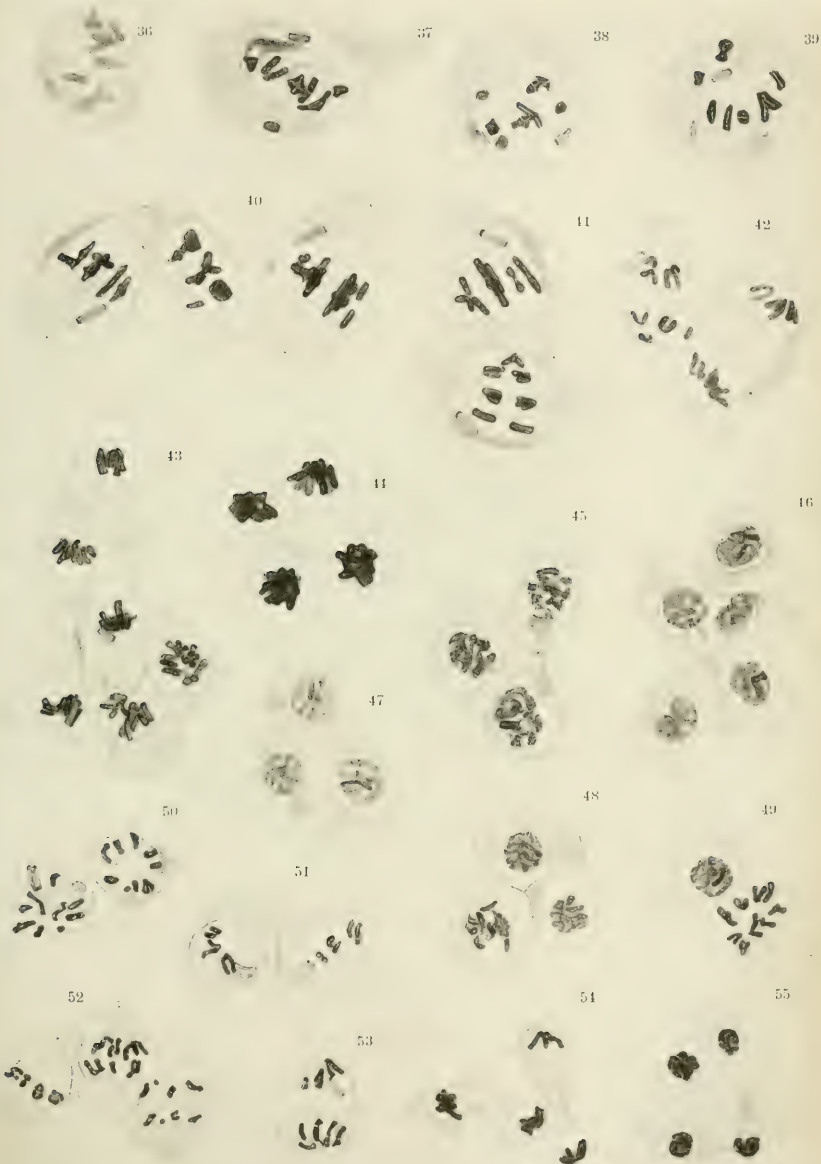
Figs. 74, 75. Prophase of the ovarian follicle cells, representing almost a like behavior to that of the spermatogonia shown in Fig. 5 and Fig. 6.

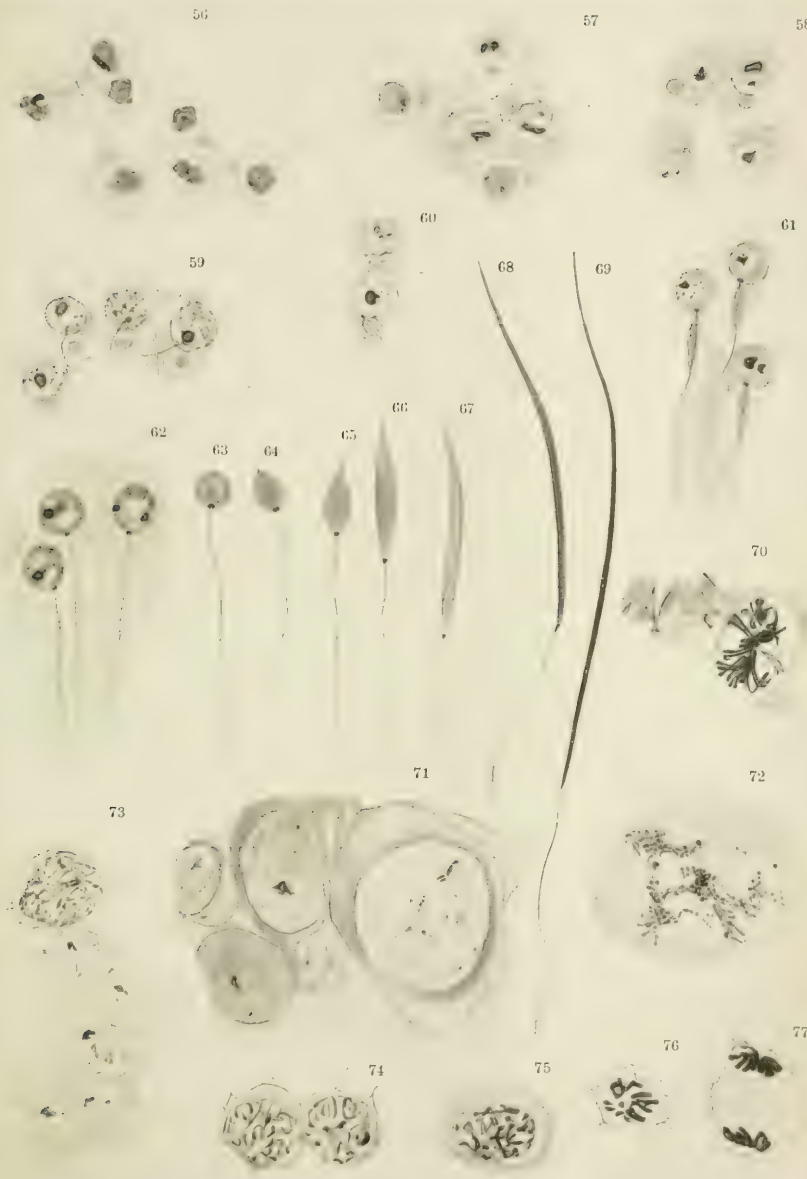
Fig. 76. Metaphase of the ovarian follicle cell.

Fig. 77. Anaphase of the ovarian follicle cell.

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Ueber das Teleskopauge des Goldfisches.

VON

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Mit Tafel XIX und 2 Textfiguren.

Das Vorhandensein eines fernrohrartig verlängerten Augenbulbus ist seit langem von vielen Naturforschern bei Wassertieren und Vögeln bestätigt worden, Dass solche Augen biologisch eine besondere Bedeutung haben müssen, kann man wohl vermuten, wenn man die Lebensweise ihrer Besitzer in Betracht zieht.

Neuerdings wissen wir aus den Arbeiten von CHUN (1887), FRANZ (1907) und insbesondere BRAUER (1908), dass die Teleskopaugen von Vögeln (z. B. Eulen), Tiefsee-Cephalopoden und -Fischen stets gleiche Funktionen haben, und zwar sehen wir sie nur unter im Dunkeln lebenden Tieren, obwohl nicht an den eigentlichen Tiefseeformen. BRAUER (1908) hat angegeben, „dass das Teleskopauge bei anderen Tieren auch schon unter dem Einfluss des Dämmerungslichtes auf dem Lande, im Süßwasser und im Meere sich ausbilden kann, aber unzweifelhaft ist es Anpassung an das Leben im Dunkeln.“ (S. 255.)

Das Teleskopauge ist also im allgemeinen eine Folge der Anpassung an äussere Bedingungen. Bei dem Goldfische ist das aber nicht der Fall. Man weiss recht gut, dass die Goldfische mit ihren so unendlich verschiedenen Formen und Merkmalen durch künstliche Zuchtwahl aus einer Urform entstanden sind, als die wir vermutlich *Carassius auratus*, L. ansehen müssen. Obwohl die Entstehung der fernrohrartig voraustragenden Augen von Goldfischen noch nicht aufgeklärt ist, können wir doch so viel sagen, dass ein

grosser Unterschied zwischen diesen und den Teleskopaugen anderer Tiere vorhanden ist.

TORNIER (1911) hat in seiner physiologisch-pathologischen Untersuchung einen sehr lehrreichen Zufall von dem Entstehungsverlaufe der abnormal vergrösserten Augen berichtet, aber er machte keinen ausführlichen Versuch hinsichtlich der Entstehung der Teleskopaugen der Goldfische. HINSEN (1913) gibt eine kurze Beschreibung über den Bau des Teleskopauges und berichtet einige neue Befunde, die aber keine zwingenden Schlüsse zulassen.

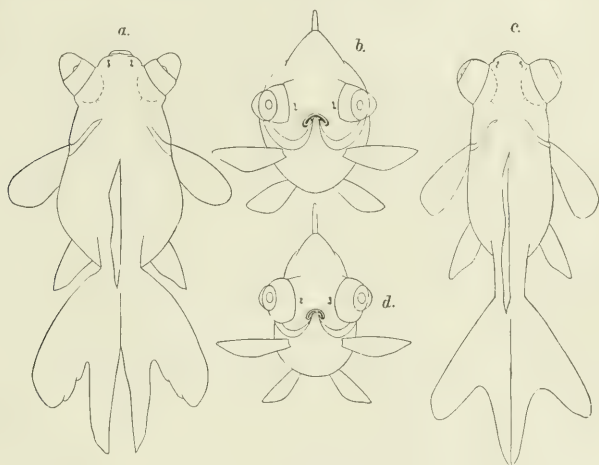
Über die verschiedenen Abarten der Goldfische mit ihren mannigfaltigen Flossenformen, Farbentönen und bizarren Gestalten werde ich keine Beschreibung geben, da dies meiner eigentlichen Untersuchung fern steht. Ich will mich darauf beschränken, die vielen morphologischen und histologischen Abweichungen und auch die verschiedenen Merkmale zwischen diesen und den allgemeinen Teleskopaugen aufzuzeigen. In Japan, wo die Goldfischzucht von vielen Leuten als Gewerbe betrieben wird, haben wir alle Gelegenheit, ansehnliche und merkwürdige Exemplare zu erhalten, und so ist ihre Untersuchung sehr erleichtert.

Aeussere Gestalt.

Wir haben zwei Hauptformen von Teleskopaugen unter den Goldfischen, eine mit seitwärts gerichteten (Textfig. 1), und eine andere mit nach oben oder zum Himmel gerichteten, sogenannten Himmelsaugen (Textfig. 2). Die seitwärts gerichteten Augen verdienen, wenn wir ihre Lage in Betracht ziehen, nicht die Bezeichnung Teleskopaugen im eigentlichen Sinne, weil beide Augen nicht parallel sind, *d. h.* die Hauptachsen (Achse der Hornhaut zum Augen Grunde) beider Augen machen irgend einen Winkel. Dagegen sind die sogenannten Himmelsaugen wahre Teleskopaugen, da ihre Hauptachsen ganz parallel nach oben gerichtet sind.

Die Form des Augenbulbus bei beiden Formen ist ebenfalls sehr mannigfaltig. Die typische Form, wenn sie auch durch die Gestalt der Cornea, wie im folgenden erwähnt, stark beeinflusst wird, ist ellipsoidisch lang, aber wir begegnen auch oft bald birnenförmigen (Textfig. 1. *c, d.*; Taf. XIX Fig. 2), bald cylinderförmigen (Textfig. 1. *a. b.*; Taf. XIX, Fig. 1).

Die Grösse, welche ausser der Gestalt und Lage äusserlich am meisten auffällt, wechselt auch sehr und zeigt grosse Schwankungen bei den Individuen im Verhältnis zur Körperlänge; daher können wir das Verhältnis des Bulbusdurchmessers zur Kopflänge u.s.w. nicht mit bestimmten Werten wie bei anderen Fischen darstellen. Aber wir können in Tabelle II. gut erkennen, dass der Bulbus im allgemeinen sehr gross ist.

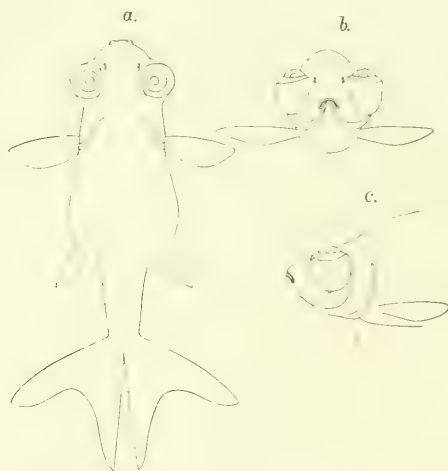


Textfig. 1. Teleskopgoldfisch. $\times 2/3$.

Die Augenhöhlen sind sehr breit und seicht, weil der kleine Schädel die grossen Augen tragen muss. Sie sind der Form des Augengrundes gemäss bei der ersten Form rund, aber beim Himmelsauge, weil die Bulbushauptachse vertikal und die Seitenwände der Bulbi (nicht die Augengründe) an dem Schädel haften, bilden sie Nischen auf beiden Seiten des Schädels, und der Bulbus ragt lateral bis zur Hälfte (oder mehr) ausserhalb der Augenhöhle hervor.

Die Fischeaugen liegen auf beider Seiten des Kopfes und ihre Hauptachsen machen einen so grossen Winkel, dass den Augen das binokulare Sehen nur in geringem Grade möglich ist. (Je kleiner der Winkel ist, desto grösser ist der Grad des binokularen Sehens, und am grössten, wenn der Winkel null ist;

d. h. die Hauptachsen parallel). Bei der vermutlichen Urform der Goldfische, *Carassius auratus* L., ist der Winkel zwischen den Hauptachsen etwa 140° — 150° , und deshalb der Grad des binokularen Sehens sehr gering; dieser Winkel ist bei den seitwärts gerichteten Teleskopaugen etwas kleiner und schwankt zwischen 110° — 130° bei den in meine Hände gekommenen Exemplaren; das binokulare Sehen wird also sehr erhöht. Die Himmelsaugen haben, wie oben



Textfig. 2. Himmelsauge. a, Dorsalansicht; b, Vorderansicht; c, Seitenansicht. $\times 2/3$.

erwähnt, die Hauptachsen parallel und der Grad des binokularen Sehens ist vollkommen.

Sowohl die Fische mit seitwärts gerichteten Teleskopaugen als auch die mit Himmelsaugen haben gleichmässig grosse Interorbitalräume, und die grossen Augenbulbus ragen halb oder mehr aus den Orbitalräumen heraus. Dieses Herausragen mag auch zu der Erweiterung des binokularen Sehfeldes vorteilhaft sein; ob es irgend eine Bedeutung für das Leben der Goldfische hat oder nicht, wissen wir nicht, aber ein solches Herausragen des Bulbus aus dem Kopfe muss zweifellos ein Hindernis für die Bewegungen des Besitzers sein.

Die Gestalt der Cornea ist sehr mannigfaltig, bald flach, bald halbkugelig, und in ausserordentlichen Fällen erhöht sich ihre Vorwölbung ungefähr kegelförmig (Taf. XIX, Fig. 1). Die Höhe der Cornea misst bisweilen $\frac{1}{3}$ der Hauptachse des Bulbus, also ist diese Vorwölbung an dem teleskopartigen Aussehen der Augen sehr beteiligt.

Die Iris bildet eine fast vertikale Wand bei dem seitwärts gerichteten Teleskopauge, und ist fast horizontal bei dem Himmelsauge zwischen der vorderen und hinteren Augenkammer. Sie ist sehr gut entwickelt, obwohl sie, wie allgemein bei den Knochenfischen, kein Vermögen der Erweiterung und Verengerung der Pupille hat. Die Iris ist im jüngeren Stadium schlecht entwickelt und die Pupille ist bei ihm verhältnismässig gross, aber mit dem Alter entwickelt sich die Iris so übermässig, dass die Pupille nicht in gleichem Grade grösser werden kann, ja wohl kleiner wird; darum ist bei volljährigen Exemplaren die Pupille sehr klein und die Linse hinten nach dem Augenrunde zu sehr gedrückt; selbstverständlich ist kein aphakischer Raum vorhanden.

Die Form der Pupille ist rund. Sie ist etwas excentrisch; bei dem seitwärts gerichteten Teleskopauge liegt sie etwas proximalwärts verschoben, bei dem Himmelsauge etwas medial. Wie es uns BRAUER's Figur veranschaulicht, liegt die Linse der Teleskopaugen bei den Tiefseefischen im allgemeinen dicht hinter der Cornea, und zwar tritt dieselbe halb oder mehr von der Ebene der Iris heraus; und weil sie sehr gross ist, nimmt sie beinahe die ganze vordere Augenkammer ein. Bei dem Teleskopauge der Goldfische ist dagegen die vordere Augenkammer sehr geräumig, teils wegen der starken Vorwölbung der Cornea, teils wegen der zurückgezogenen Lage der Linse.

Der Opticus tritt fast an derselben Stelle des Augengrundes ein wie bei *Carassius auratus*, das heisst, er verschiebt sich ein wenig caudal von dem Mittelpunkt des Augengrundes. Während bei dem seitwärts gerichteten Teleskopauge der Opticusnerv gerade vom Gehirn zum Bulbus läuft, kann er beim Himmelsauge nicht gerade zum Augenrunde gehen, weil die proximale Augenbulbuswand eine so starke Ausdehnung erlitten hat, dass es scheint, als ob der Bulbus um 90° sich vertikal nach oben gedreht habe, und sich noch dazu nach unten weiter als das Niveau der Ursprungsstelle des Opticus erstreckt. Er läuft also um eine beträchtliche Strecke der Bulbuswand

entlang und tritt in den Bulbus an einem etwas lateralwärts verschobenen Punkte ein (Taf. XIX, Fig. 3—5).

Augenmuskeln.

Die Augenmuskeln zeigen beim seitwärts gerichteten Teleskopauge die für ein typisches Wirbeltierauge charakteristische Anordnung, indem die zwei *M. obliqui* etwas besser entwickelt sind als die vier *M. recti*.

Beim Himmelsauge, wo der Augenbulbus eine Verlagerung erlitten hat, haben die Augenmuskeln eine Lageveränderung erfahren. Trotzdem sind sechs Muskeln vorhanden wie bei dem normalen; sie verhalten sich aber ganz anders. Keiner von den sechs Augenmuskeln kann das Auge seitwärts richten, weil keiner an der lateralen Wand des Bulbus sitzt.

Wie die Abbildungen (Taf. XIX, Fig. 3—5) uns erkennen lassen, sitzen alle Muskeln an der proximalen Bulbuswand, welche eigentlich die dorsale Wand wäre und durch die ungleichmässige Ausdehnung der Bulbuswand zur proximalen geworden ist. Nur zwei Muskeln, *M. rectus superior*, *M. obliquus superior* haben ihre eigentliche Lage behalten; die anderen vier schieben sich nach der proximalen Wand zusammen. *M. rectus inferior* und *M. obliquus inferior* legen sich dicht aneinander und sitzen nicht an der lateralen Wand, welche eigentlich die ventrale Wand ist. Sie ändern ihre Plätze und sitzen an der proximalen Seite der Wand (Taf. XIX, Fig. 3). Infolgedessen sitzen zwei *obliqui* an der proximalen Wandhälfte des Bulbus, was nicht der Fall für ein typisches Wirbeltierauge ist. *M. rectus anterior*, der am besten entwickelte, sitzt an der mittleren Region der proximalen Wand. *M. rectus posterior* ebenfalls etwas proximalwärts verschoben und nähert sich dem *M. rectus superior*. Man kann durch die Lage der Muskeln sich leicht vorstellen, dass für den Fisch mit Himmelsaugen das Gesichtsfeld sehr beschränkt oder verkleinert ist. Die Augen können jedoch ziemlich gut vorwärts gerichtet werden, da vier Muskeln (zwei *obliqui*, *rectus inferior*, und wahrscheinlich *rectus anterior*) dafür vorhanden sind (Taf. XIX, Fig. 4).

Die Augenwand.

Die Bulbuswand des Teleskopauges ist ausserordentlich dünn gebildet ;

dieses Phänomen ist, wie HIRSCH (1913) meinte, als eine Folge der Vergrößerung des ganzen Bulbus anzusehen. Eine dünne Membran, welche sich zur Stütze der Cornea ausgebildet hat, überdeckt diese. Sie ist eine Fortsetzung der Körperoberhaut, die hier dünn und durchsichtig geworden ist. Die Sclera ist von zwei Teilen gebildet; der Scleraknorpel ist dünn und deckt nur den distalen Teil des Bulbus, das Sclerabindgewebe umhüllt nur den Augengrund (Taf. XIX, Fig. 1, 2).

Die Chorioidea ist auch dünn. Sie enthält nur wenige Gefässe. Die sogenannte Chorioidealdrüse oder Rete mirabile ist sehr rückgebildet, obwohl sie noch ihre eigentliche Hufeisenform hat und reichliche Pigmente enthält. Von ihr müssen wir annehmen, dass eine grosse Rückbildung stattgefunden hat, weil wir sie bei *Carassius auratus* sehr kräftig entwickelt finden. Ligamentum pectinatum (Taf. XIX, Fig. 1, 2. *Lig. pect.*) ist sehr gut entwickelt, wie bei *Carassius*.

Die Retina.

BRAUER (1908) hat dargelegt, dass einer der charakteristischen immer wiederkehrenden Züge für das Teleskopauge die Teilung der Retina in eine Haupt- und Nebenretina ist. Dies gilt jedoch nicht für den Goldfisch; die Teilung der Retina findet hier nicht statt. Die Opticuspupille liegt beim Goldfische im Augengrunde, nicht in der seitlichen Augenwand, wie bei den Tiefseefischen.

Die am meisten auffallende Eigentümlichkeit dieses Teleskopauges ist die Rückbildung der Retina in einem Teile des Augengrundes. Im Widersprache mit HIRSCHS Behauptung verschwindet, wie die Abbildungen (Taf. XIX, Fig. 1, 2) uns erkennen lassen, die Zapfen- und Stäbchenschicht der Retina in dem Augengrunde, und nur das Retinapigment bleibt zurück. Die innere molekuläre Schicht ist noch in diesem Teile vorhanden, wo die anderen Schichten sich schon rückgebildet haben, aber in der Nähe der Opticuspupille verschwindet auch diese Schicht (Taf. XIX, Fig. 1, 2), was eine Erscheinung ist, der wir niemals bei anderen Fischen begegnen. Bei *Carassius auratus* ist die Dicke der Retina, besonders die der Zapfen- und Stäbchenschicht, in der Nähe der Opticuspupille am grössten und nimmt nach der Iriswurzel allmählich

ab. Dagegen ist bei diesem Teleskopauge die Dicke in der seitlichen Augenwand am grössten, und auch an der Iriswurzel nimmt sie nicht beträchtlich ab. Ich lasse hier eine Tabelle folgen, welche die Dicken jeder Schicht der Retina zeigt.

TABELLE I.

		Pigmentepithelschicht. Zapfen- und Stäbchen- schicht.	Äussere Körnerschicht.	Äussere granulirte Schicht.	Innere Körnerschicht.	Innere granulirte Schicht.	Ganglienzellschicht.	Nervenfaserschicht.	Breite der Retina. (Nervenfaserschicht ausgenommen.)
<i>C. auratus</i> Kopflänge 72mm	an Augengrunde.	65 μ	25 μ	15 μ	10 μ	65 μ	25 μ	35 μ	235 μ
	an der Seitenwand.	50	20	15	40	55	10	10	190
Goldfisch Kopflänge 62mm	an der Seitenwand.	50	15	5	35	20	5	20	170
	in der Nähe der Iriswurzel.	45	15	5	35	20	5	5	125

Aus dieser Tabelle kann man leicht erkennen, dass die Retina im Teleskopauge des Goldfisches im ganzen sich rückgebildet hat. Selbst an dem am besten entwickelten Teile, d. h. an der Seitenwand, ist die Retina noch dünner als die am Augengrunde dieses letzteren. Die Rückbildung, wie oben erwähnt, ist am stärksten am Augengrunde, wo schon keine Elemente der Lichtempfindung des Gewebes mehr vorhanden sind und nur das Pigment und die innere granulirte Schicht übrig bleiben. Wenn man dieses Verhalten mit der Retina der Tiefseeteleskopfische vergleicht, so kann man gleich einen grossen Unterschied zwischen den beiden bemerken. Bei Tiefseeteleskopfischen ist die Retina an der Augenwand niemals rückgebildet, ja vielmehr stark entwickelt, wie BRAUER uns gezeigt hat.

Das zurückbleibende Pigment der rückgebildeten Retina wurde bereits von RITTER (1893) bei *Typhlogobius* und von EIGENMANN (1899) bei amerikanischen blinden Wirbeltieren, *Amblyopsidae*, deutlich erklärt. RITTER erklärt bei *Typhlogobius*: „An increase of pigment is an incident to the gradual diminution in functional importance.“ EIGENMANN sagt auch: „primary the pigment layer has retained its normal condition, while the rest of the retina

has been simplified, and there may be even an increase in the thickness of the layer“ (S. 590), und auch „usually to be found in degenerate eyes“ (S. 551). Die Pigmentschicht zeigt sich hier als eine Fortsetzung des Pigmentepithels der Retina, darum können wir mit einem Blick erkennen, was der Ursprung der zurückbleibenden Pigmentschicht ist, obgleich die Zunahme des Pigmentes nicht zu sehen ist wie bei den *Amblyopsidae* (Taf. XIX, Fig. 6—8).

EIGENMANN bemerkte auch bereits das Zurückbleiben der inneren granulierten Schicht bei blinden Fischen, und schreibt dazu: diese Schicht „owes its retention more to its supporting than to its nervous elements“ (S. 692). Wie die Abbildung zeigt (Taf. XIX, Fig. 6—8), bleibt auch in diesem Falle die innere granulierten Schicht in dem rückgebildeten Teile der Retina. Das Vorhandensein der kräftigen Nervenfaserschicht in demselben Teile ist wohl selbstverständlich, weil sie eine Nervenbahn vom Opticus zu den nutzbaren Retinaclementen bildet. Wie die Tabelle I. uns erkennen lässt, ist die Breite der Retina beim Teleskopgoldfische ja im höchst entwickelten Teile noch dünner als die der Augenseitenwand der vermutlichen Urform. Insbesondere die äussere Körnerschicht, äussere granulierten Schicht und innere granulierten Schicht sind sehr reduciert. Die Kerne der verschiedenen Arten sind auch mehr zerstreut in der Retina des Goldfisches (Taf. XIX, Fig. 6—8), auch sind bei ihm beide Elemente, die Zapfen und Stäbchen, gut entwickelt, was bei dem gewöhnlichen Teleskopauge niemals der Fall ist; bei Tiefseeformen fehlen die Stäbchen ganz als eine Folge der Anpassung ans Dunkelleben.

Ueber das dioptrische Verhalten.

Wie BRAUER uns erkennen lässt, hat das Teleskopauge von Tiefseefischen sich zur Anpassung ans Dunkelleben ausgebildet, bei dem Goldfische ist es, wie oben erwähnt, ganz anders. Der dioptrische Unterschied zwischen den beiden Augen ist daher sehr gross.

Die Cornea, wenn sie auch grosse Verschiedenheiten in der Grösse und Gestalt bei Individuen zeigt, hat im allgemeinen sehr starke Vorwölbung. In ausserordentlichen Fällen steht sie asymmetrisch zapfenförmig empor (Taf. XIX, Fig. 1). Bei einem solchen Exemplar muss die Brechung des Lichtes durch die Cornea sehr stark sein.

Die Linse, welche von der Cornea weit entfernt liegt, ist auffallend klein (Taf. XIX, Fig. 1, 2), aber da die Pupille sehr eng ist, bleibt der aphakische Raum nicht bestehen. Wie bei *Carassius auratus*, sind Processus falciformis und Campanula halleri (Retraktor der Linse) vorhanden, jedoch konnte ich bei manchen Exemplaren Processus falciformis nicht finden. Der Retraktor der Linse ist sehr klein und in manchen Fällen hat er sich zu einer nur kleinen Pigmentmasse rückgebildet. In dieser Hinsicht steht meine Beobachtung der HIRSCH'schen entgegen; die Zahl der von diesem Autor benützten Exemplare war vielleicht zu klein, um einen richtigen Schluss zu ergeben.

Die Form der Linse ist normal, also kugelig. Wir verdanken L. MATTHIESSEN (1882) die Kenntnis, dass bei den Fischen die relative Brechkraft, d. h. die Brennweite, im Verhältnisse zum Linsendurchmesser konstant ist. Bei allen von ihm untersuchten Fischen zeigt das Verhältnis des Retinaabstandes zum Linsendurchmesser einen konstanten Wert, und zwar durchschnittlich 1:2,52. FRANZ (1907) erweiterte diese Erkenntnis und bestätigte, dass diese von MATTHIESSEN gefundene Proportion auch bei den Schachiaugen (bei fixierten; daher der Schrumpfung ausgesetzten Schachiaugen 1:2,4) vorhanden ist. Darum „muss die Fischlinse stets eine ganz bestimmte Brechkraft oder »Totalindex« haben und zwar einen solchen, dass sie bei einem Radius r^{mm} die Lichtstrahlen in etwa $2,52 \times r^{\text{mm}}$ Abstand von ihrem Zentrum sammelt“ (S. 276). Er bestätigte diese Tatsache auch bei den Tiefseefischen und formulierte den Satz, dass „die Teleskopaugen der Tiefseefische gleich den Augen der Flachseefische auf deutliche Schwerte eingestellt sind“ (S. 277).

Auch BRAUER (1908) hat festgestellt, dass die von der Valdivia-Expedition gesammelten und von ihm untersuchten Teleskopfische den gleichen Modus in bezug auf den Totalindex haben, wenn ihre Augen auch röhrenförmig gestaltet sind. Sie haben sehr grosse Linsen und dementsprechend ist der Abstand vom Linsenzentrum zur Hauptretina so gross, dass das Verhältnis des Abstandes zum Linsendurchmesser nicht verändert ist. Der Zahlenwert des Verhältnisses ist nach ihm in Wirklichkeit „derselbe wie beim Seitenauge, es schwankt nämlich zwischen 1:2 und 1:2,8, im Durchschnitt beträgt es 1:2,3, also, wenn man die infolge der Konservierung notwendig eingetretenen Veränderungen berücksichtigt, wie beim normalen Seitenauge“ (S. 243). Wir wissen ferner sowohl von FRANZ (S. 276) als auch von BRAUER (S. 253), dass

alle Teleskopaugen mit wohl entwickeltem Retraktor der Linse versehen sind und damit vollständiges Akkommodationsvermögen besitzen.

Das dioptrische Verhalten ist beim Goldfischteleskopauge sehr verändert. Hier haben wir keinen wohl entwickelten Retraktor der Linse, welcher den Akkommodationsvorgang ermöglicht. Die Linse selbst ist sehr klein, und die Retina als eine rückgebildete Form liegt im Augen Grunde sehr entfernt vom Linsenzentrum. Die nachfolgende Tabelle II zeigt das Verhältnis, welches uns das dioptrische Verhalten bei einigen am meisten ausgebildeten typischen Exemplaren erklärt. Äusserlich ist bei dem ersten von ihnen der Bulbus merklich cylindrisch, bei den anderen ellipsoidisch oder oval. Das Verhältnis des Retinaabstandes zum Linsendurchmesser ist ausserordentlich gross, wie Tabelle II zeigt.

TABELLE II.

Körperlänge ausschliesslich d. Schwanzes.	Kopflänge.	Hauptachse des Bulbus.	Grösster Durchmesser d. Bulbus.	Abstand d. Iris zur Retina im Augen- grunde.	Linsendurchmesser.	Abstand d. Retina im Augengrunde vom Linsenzentrum.	Verhältnis d. Retina- abstandes zum Linsenzentrum.
64mm	29mm	14,0mm	6,0mm	10,5mm	2,9mm	9,5mm	1 : 3,27
68	31	13,5	10,0	10,1	2,8	9,1	1 : 3,25
68	31	13,3	9,5	10,2	2,8	8,8	1 : 3,14
65	29	11,2	9,5	9,2	2,7	7,9	1 : 2,92

Die Tatsachen, dass diese Zahlenwerte der Verhältnisse gross sind und die Linsen keinen wirklichen Retraktor haben, ermächtigen uns das Fehlen des Akkommodationsvermögens für die Teleskopaugen des Goldfisches anzunehmen. Die starke Vorwölbung der Cornea lässt auch das Bild notwendigerweise näher projicieren. Aus solchem Grunde, wemgleich die Retina im Augengrunde wohl entwickelt ist, wird wahrscheinlich das Bild von ihr nicht empfangen. Aber der Abstand der Retina vom Augengrunde nach der Iriswurzel nimmt allmählich ab, und der Zahlenwert (Retinaabstand durch Linsendurchmesser dividiert) wird dementsprechend kleiner; das schräg in die

hintere Augenkammer hineinkommende Licht kann das Bild auf der Retina in irgend einer Gegend der Augenseitenwand projizieren. TORNIER (1911) konstatierte schon bei pathologisch abnormal vergrößerten Augen eine Art von Retinarückbildung, die stellenweise, nicht in bestimmten Stellen stattfindet. Bei Goldfischteleskopaugen ist die Rückbildung oder Atrophie der Retina immer nur am Augengrunde zu beobachten.

Die biologische Bedeutung der Rückbildung der Retina im Augengrunde wird uns klar, wenn wir das dioptrische Verhalten dieser Augen in Betracht ziehen. Die Retina im Augengrunde liegt zu entfernt vom Linsenzentrum, um das Bild zu empfangen, daher ist ihr Vorhandensein nicht mehr notwendig, und so hat sie sich rückgebildet. An der Augenseitenwand wird dagegen der Abstand der Retina vom Augengrunde nach der Iriswurzel zu allmählich verkürzt, und so liegt die Retina hier in verschiedener Entfernung vom Linsenzentrum. Sie ist hier bei mässiger Entfernung der Ikono- und Moto-reception dienstbar, so hat sich ihre Entwicklung erhalten, wenngleich eine hochgradige Akkommodation im Leben des von Menschen gezüchteten Goldfisches nicht nötig sein dürfte.

Zusammenfassung.

1. Während das gewöhnliche Teleskopauge eine Folge der Anpassung ans Dunkelleben ist, ist es bei dem Goldfische vermutlich durch künstliche Zuchtwahl des Menschen entstanden.

2. Das Fehlen der seitlichen Ausbauchungen gibt dem Teleskopauge des Tiefseefisches eine fernrohrartige Form (nach FRANZ); die Röhrenform ist also eine passive Erscheinung. Beim Goldfische dagegen ist es eine Folge des übermässigen Wachstums der Wand, dass der Bulbus eine Röhrenform bekommt.

3. Die Vorderaugenkammer ist hier sehr geräumig, während bei dem allgemeinen Teleskopauge die Linse den ganzen Raum einnimmt.

4. Die für ein typisches Wirbeltierauge charakteristischen Augenmuskeln sind alle vorhanden, obwohl sie beim Himmelsauge eine Verlagerung erfahren.

5. Die Eintrittsstelle des N. opticus ist normal; nur beim Himmelsauge ist ihre Lageveränderung die Folge des ungleichmässigen Wandwachstums,

aber nicht in so hohem Grade, wie sie von BRAUER bei Tiefseeteleskopfischen konstatiert wurde.

6. Im Gegensatz zu dem gemeinen Teleskopauge zeigt sich hier eine wohl entwickelte Iris, keine Linse und grosser Retinaabstand. Der Retraktor der Linse und Processus falciformis haben sich sehr rückgebildet. Darum besitzt das Goldfischteleskopauge keinen wirklichen Akkommodationsapparat im Innern des Auges.

7. Die Retina im Augengrunde hat sich rückgebildet, weil es hier keine Möglichkeit zur Bildwahrnehmung gibt. Dieser Punkt ist ein erheblicher Unterschied zwischen den Augen des Teleskopgoldfisches und denen des dem Dunkel sich anpassenden Tiefseeteleskopfisches, bei welchem die Retina am Augengrunde am höchsten ausgebildet ist. Die Lichtstellung des Pigmentes und das Vorhandensein von Zapfen mit Stäbchen in der Retina des Goldfischteleskopauges sind nicht der wesentliche Unterschied; sie werden uns klar, wenn wir daran denken, dass der Goldfisch im Lichte lebt.

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TAFELERKLÄRUNG.

<i>Bl.</i>	Blutkörperchen.	<i>Rückg. Teil. d. Retina.</i>	
<i>C.</i>	Cornea.		Rückgebildete Teil der Retina.
<i>Chor.</i>	Chorioidea.		
<i>Chor. dr.</i>	Chorioidealdrüse.	<i>R. a.</i>	M. rectus anterior.
<i>H.</i>	Körperoberhaut.	<i>R. i.</i>	M. rectus inferior.
<i>Lig. pect.</i>	Ligamentum pectinatum.	<i>R. p.</i>	M. rectus posterior.
<i>Lig. s. l.</i>	Ligamentum suspensorium	<i>R. s.</i>	M. rectus superior.
	lentic.	<i>Scl.</i>	Sclera.
<i>N. op.</i>	Nervus opticus.	<i>Scl. Knor.</i>	Scleraknorpel.
<i>Ob. i.</i>	M. obliquus inferior.	<i>2, 3, 4, 6.</i>	Zweiter, dritter, vierter und
<i>Ob. s.</i>	M. obliquus superior.		sechster Hirnnerv.

TAFEL XIX.

- Fig. 1. Augenbulbus des cylinderförmigen Typus.
 Fig. 2. Augenbulbus des birnenförmigen Typus.
 Fig. 3. Das linke Auge des Himmelsauges; von innen gesehen.
 Fig. 4. " " " " " ; von vorn gesehen.
 Fig. 5. " " " " " ; von hinten gesehen.
 Fig. 6. Retina der Augenseitenwand.
 Fig. 7. Retina des Augengrundes.
 Fig. 8. " " " nahe bei der Opticus Eintrittsstelle.

BERICHTIGUNG.

Tafel XIX, Fig. 2: Statt *choe* lies *chor*.

1.

Lip. sl.
H.

Scl. knor.

Lip. post

Scl. knor.

Scl.

Scl.

Chor. dr.

Rückg. Teil d. Retina

2.

Lip. post

H.

Chor.

Chor. dr.

Rückg. Teil d. Retina.

3.

4.

R. s.

2

R. p

R. a. 6

R. i. 3

R. s. 3

R. p 6

R. i.

Ob. i.

R. a.

2

6.

Ob. s.

7.

Chor.

Bl.

R. s.

R. .

R. p

Ob. i.

6

3

R. a

Ob. s.

4

S.

Chor.

Bl.



Descriptions of Four New Species of Lepidoptera Heterocera from Japan.

By

Nobukatsu Marumo.

With Plate XX.

NOCTUIDÆ.

Mythimna rufomedialis n. sp.

(Pl. XX, figs. 3-5.)

Head and thorax purplish brown mixed with white; palpi dark brown; pectus grey-brown; legs dark brown tinged with purplish, tarsi ringed with white; abdomen grey-brown, the ventral side and the anal tuft tinged with purplish red. Forewing grey, irrorated with rufous; an indistinct subbasal line from costa to vein 1; a waved dark antemedial line; orbicular whitish grey, its inner angled outwards at middle, defined by black and tinged with rufous before it in cell; claviform oblong, defined by black, tinged with rufous; reniform whitish grey, tinged with rufous on its upper part, and defined by black, the space between it and the orbicular blackish tinged with rufous; an indistinct oblique shade from lower end of reniform to inner margin, with rufous tinge on it and connected by a rufous streak with claviform; postmedial line black, dentate, defined by whitish grey on its outer side, bent outwards below costa, incurved below vein 3; an indistinct subterminal line, tinged with rufous; a terminal series of black lunules; cilia grey-brown, slightly tinged with pink, a whitish line at base. Hindwing whitish, slightly tinged with pink and irrorated with fuscous; a dark terminal line; cilia whitish, tinged with pink. Underside of forewing brown, tinged

with purplish red on costal and terminal areas; a dark discocellular spot and a dark postmedial line; of hindwing whitish tinged with purplish red; a dark discocellular spot and a slightly waved postmedial line.

Expanse: 38-40 mm.

A male type in my collection, taken by Mr. YAZAWA on Mt. Shirouma (about 3,000 m.), August 7, 1916. Another male specimen taken also by Mr. YAZAWA.

Comes near to *M. rubricosa* Schiff., but readily distinguished from it by the rufous tinge at middle of forewing.

Hyposada otoensis n. sp.

(Pl. XX, fig. 2.)

Head, palpi and tegulae dark brown, mixed with whitish; thorax and abdomen pale brown, irrorated with dark brown and black; forefemur with black hair. Forewing pale ochreous, irrorated with brown and crimson; costal margin fuscous; antemedial line very indistinct; a dark spot at end of cell; postmedial line double, bent outwards below costa, then oblique and slightly waved, with a dark triangular large patch on its outer side at costa; the area beyond it, except towards costa, suffused with brown; a fine waved blackish terminal line, with a series of black points on it in the interspaces of veins; cilia whitish, mixed with brown and crimson. Hindwing pale ochreous, irrorated with brown and crimson; inner area paler; a dark antemedial line almost straight, obsolescent towards costa; a blackish postmedial line excurved at middle, incurved between veins 5 and 6 and again on submedian fold; subterminal line very indistinct, slightly waved; a fine waved blackish terminal line with a series of black points on it in the interspaces of veins; cilia as in forewing. Underside whitish, irrorated with brown; forewing with the costa ochreous, suffused with brown on terminal area, traces of a curved postmedial line; hindwing with a dark discocellular spot and traces of a postmedial and subterminal curved lines; an indistinct terminal series of dark spots on both wings.

Expanse: 20 mm.

A male type in my collection, taken by me at Komori, Yamato, August 5, 1916.

LASIOCAMPIDÆ.

Takanea japonensis n. sp.

(Pl. XX, fig. 1.)

Head, thorax and abdomen red-brown, tegulæ with a chestnut or crimson longitudinal stripe at middle. Forewing red-brown; veins red-brown or crimson, streaked with black on them at middle; antemedial line dark, bent outwards below costa and on median nervure, the area inside of it white and tinged with red-brown; a black spot on discocellulars; postmedial line pale ochreous, indistinct, bent outwards below costa, then oblique and slightly sinuous to vein 2 and angled inwards on vein 1; subterminal line pale ochreous, somewhat dentate, excurved between veins 6 and 8, and again between veins 3 and 4, the area beyond it above vein 6 suffused with fuscous; cilia red-brown spotted with blackish. Hindwing brown; veins streaked with red-brown; antemedial line pale and indistinct, arising from the white spot on costa; cilia red-brown, spotted with blackish. Underside of forewing mostly like the upper, the postmedial line distinctly defined on inner side by red-brown; of hindwing reddish brown, with the costa dark red-brown; antemedial line dark red-brown, defined by white on its outer side, angled inwards on vein 2 and not reaching inner margin; an indistinct pale postmedial line, excurved at middle, defined slightly on both sides by red-brown.

Expanse: 46 mm.

A male type in my collection, taken by me at Dorokawa, Yamato, August 8, 1916. Another male by Mr. YANO at Nikko, July, 1916.

Comes near to *T. (Crinoeraspeda?) excisa* Wileman from Formosa and the present species may possibly be a form of it.

GEOMETRIDÆ.

Sarcinodes? mongaku n. sp.

(Pl. XX, fig. 6-8.)

Head and tegulæ grey-brown, mixed with white; palpi whitish grey, irrorated with brown; thorax and abdomen red-brown, the latter irrorated

with black; femora and tibiae white, tinged with pink and irrorated with black, tarsi fuscous on outer side. Forewing red-brown suffused with pink and irrorated with black; veins finely streaked with white; costal area crimson; an antemedial black spot on costa; a black spot on discocellulars; an oblique dark olive line from apex to inner margin beyond middle, defined by white on inner side and slightly sinuous above vein 6; an indistinct subterminal series of dark spots; cilia dark brown. Hindwing red-brown, its basal half paler, suffused with pink and irrorated with black; veins streaked finely with white; an almost straight postmedial dark olive line, somewhat diffused outwardly, edged with white on its inner side; cilia dark brown. Underside pinkish white, suffused with dark olive and red, and irrorated with fuscous; forewing with a black spot on discocellulars; a postmedial series of black spots on veins and a very indistinct series of dark spots just before termen; hindwing with an indistinct dark spot on discocellulars, an irregular indistinct pale subterminal line, the area beyond it dark olive.

Expanse: 46 mm.

A male type in my collection, taken by me at Nachi, Kii, July 28, 1916.

In the present species and *Sarcinodes restitutaria* the vein 9 arises from 10 and anastomoses with 8. It seems that it is better to remove those two species from the genus *Sarcinodes*.

EXPLANATION OF PLATE XX.

- Fig. 1. *Takamea japonensis*, ♂.
 Fig. 2. *Hyposada otoensis*, ♂, ×1.5.
 Fig. 3. *Mythimna rufomedialis*, ♂.
 Fig. 4. Head of ditto.
 Fig. 5. Wings of ditto.
 Fig. 6. *Sarcinodes? mongaku*, ♂.
 Fig. 7. Head of ditto.
 Fig. 8. Wings of ditto.



A Revision of the Japanese Pyralidæ.

Part I (Subfamily Epipaschiinæ¹).

By

Nobukatsu Marumo.

With Plate XXI.

The Epipaschiinæ together with the other subfamilies, Endotrichinæ, Pyralinæ, and Chrysauginæ² belongs to the group of the Pyralidæ in which the median nervure of the hindwing is non-pectinate on upperside, and the vein 7 of the forewing stalked with 8 and 9. The Epipaschiinæ is readily distinguished from the other three subfamilies by having the small tufts of scales in the forewing in the cell and on the discocellulars.

Nineteen species of the Epipaschiinæ which have been recorded from Japan are distributed amongst the five genera, *Arnatula*, *Maceda*, *Locastra*, *Stericta*, and *Orthaga*. It seems, however, to me that to these one more is to be added, since there is a group of species which are to be referred to none of these 5 genera.

This group of species includes the forms in relatively small size and has a close affinity with the genus *Lepitogma*, differing slightly from it. But the difference between the two groups is so slight that I sink the former into the latter in this paper.

In the present paper a species new to science is described.

The species not examined by me are marked with *.

KEY TO THE GENERA.

- A. Hindwing with vein 8 anastomosing with vein 7.
 - a. Hindwing with veins 4 and 5 stalked 1. *Arnatula*.
 - b. Hindwing with veins 4 and 5 approximated for a short distance..... 2. *Lepitogma*.
- 1. The subfamily name should be amended to *Pococerinae*.
- 2. *Chrysauginæ* should be amended to *Signinae*.

B. Hindwing with vein 8 free.

- a. Forewing with veins 4 and 5 approximated for about $\frac{1}{2}$ length; the discocellulars biangled. 3. *Macalla*.
- b. Forewing with veins 4 and 5 not approximated towards base; the discocellulars not biangled.
 - a'. Palpi with the second joint reaching well above vertex of head 4. *Stericta*.
 - b'. Palpi with the second joint hardly reaching vertex of head.
 - a². Tibiæ and tarsi nearly smoothly scaled. 6. *Orthaga*.
 - b². Tibiæ and tarsi fringed with long hair. 5. *Locastra*.

Genus ARNATULA.

Arnatula Standinger; Hampson, Trans. Ent. Soc. 1896, p. 455.

1. *Arnatula melanophila*.

Noctuides melanophila Standinger, Iris. V. p. 466, pl. III. f. 22 (1892).

Arnatula melanophila Standinger, Iris. VI. p. 78 (1893).

Parorthaga euryptera Meyrick, Trans. Ent. Soc. Lond. 1894, p. 476.

Loc.: Honshiu (Nagahama, Kyoto); Siberia.

Time of appearance: July.

Genus LEPIDOGMA.

Lepidogma Meyrick; Hampson, Trans. Ent. Soc. Lond. 1896, p. 459.

2. *Lepidogma japonica* n. n.¹

(Pl. XXI, fig. 13.)

Orthaga basalis South, Trans. Ent. Soc. Lond. 1901, p. 417.

Loc.: Honshiu (Tokyo); Kiushiu; Corea.

Time of appearance: June, July.

The name *Orthaga basalis* is preoccupied, so that the new name should be selected.

3. *Lepidogma kiiensis* n. sp.

(Pl. XXI, figs. 14, 15, 16.)

Palpi and frons black mixed with grey; the processes which arise from

1. *Lepidogma melanobasis* Hampsn. [A.M.N.H. (7) XVII. p. 129 (1906)] is probably identical with this species.

the base of antennæ whitish grey mixed with black and ochreous; vertex of head and thorax ochreous, the patagia tipped with black; abdomen ochreous so thickly irrorated with black that the ground colour is hardly visible except on basal segments; pectus, abdomen beneath, and legs except tarsi white irrorated with black; tarsi black ringed with white. Forewing olive yellow, with the costa tinged with black; a spot in and below cell near base; an antemedial spot on costa and on inner margin, the spot on inner margin forming a line; a black spot on discocellulæ; postmedial line black, minutely dentate, expanded and forming a broad band below vein 5; terminal area suffused with black; a terminal series of black spots; cilia fuscous with a dark line near base. Hindwing fuscous; cilia as in forewing. Underside fuscous with traces of postmedial line.

In one male specimen the forewing wholly suffused with blackish, leaving a pale costal fascia, and the discoidal mark being only distinct.

Expanse: 16–20 mm.

Four male specimens taken at Nachi, Kii, on 19th and 25th, July 1917.

Genus MACALLA.

Macalla Walker; Hampson, Trans. Ent. Soc. Lond. 1896, p. 463.

The veins 4 and 5 of the hindwing have been overestimated by Hampson. These two veins are closely approximated towards origin in some specimens, but not so in the others.

4. *Macalla moncusalis*.

(Pl. XXI, figs. 1, 2.)

Lamida moncusalis Walker, Cat. XVI. p. 252 (1858).

Allata penicillata Walker, Cat. XXVII. p. 111 (1863).

Orthaga obscura Moore, Lep. Atk. p. 201 (1887).

Macalla moncusalis Hampson, Moths Ind. IV. p. 113 (1896).

Loc.: Honshiu (Kaga, Tokyo); Kiushiu (Bungo, Satsuma).

Time of appearance: June, August.

Hampson places this species together with the followings under his Sect. I, in which the male has "the 2nd joint of palpi short, the 3rd immensely

dilated and curved over vertex of head with a hollow containing the maxillary palpi which are tufted with long hair; paired tufts of hair behind antennæ; " but in the specimens identified by me with such species as *moncusalis*, *amica* and *nigrescens*, the 2nd joint of the palpi immensely dilated with the hollow containing the brush-like maxillary palpi, the 3rd minute instead of being dilated, and without processes behind antennæ.

5. *Macalla amica*.

(Pl. XXI, figs. 3, 4, 5.)

Locastra amica Butler, A. M. N. H. (5) IV, p. 447 (1879).

Macalla amica Hampson, Trans. Ent. Soc. Lond. 1896, p. 464.

Loc.: Honshiu (Tokyo, Kii, Kaga, Yokohama); Shikoku (Iyo).

Time of appearance: July, August.

In most specimens the veins 6 and 7 of the hindwing are stalked.

6. *Macalla nigrescens*.

Parasarama (?) *nigrescens* Warren, A. M. N. H. (6) VII, p. 428 (1891).

Macalla nigrescens Hampson, Trans. Ent. Soc. Lond. 1896, p. 464.

Loc.: Hokkaido (Sapporo); Kiushiu.

Time of appearance:

I have received a male specimen from Isshiki, in which the expanse much smaller (21 mm.) than the type (26 mm.).

7. *Macalla margarita*.

Locastra margarita Butler, Ill. Typ. Het. III, p. 66, pl. 57, f. 4 (1879).

Locastra lativitta Moore, Lep. Atk. p. 199, pl. 7, f. 1 (1887).

Macalla margarita Hampson, Moths Ind. IV, p. 116 (1896).

Loc.: Honshiu (Tokyo, Kii, Yokohama); India; Borneo.

Time of appearance: July.

Belongs to HAMPSON'S Sect. V (incorrectly indicated as IV).

8. *Macalla inimica*.

(Pl. XXI, fig. 7.)

Locastra inimica Butler, A. M. N. H. (5) IV, p. 448 (1879). *

Pseudolocastra inimica Warren, A. M. N. H. (6) VII. p. 429 (1891).

Macalla inimica Hampson, Trans. Ent. Soc. Lond. 1896, p. 464.

Loc.: Honshiu (Tokyo, Kyoto); Kiushiu.

Time of appearance: September.

HAMPSON places this species in his Sect. I, but in the specimen examined and identified with this species by me the palpi of the male similar to those of the female and without paired tufts of hair behind the antennæ. It is, therefore, natural to remove this species from Sect. I to Sect. V.

*9. *Macalla bilineata*.

Macalla bilineata Wileman, Trans. Ent. Soc. Lond. 1911, p. 364.

Loc.: Honshiu (Settsu).

Time of appearance: July.

*10. *Macalla elegans*.

Macalla elegans Butler, Trans. Ent. Soc. 1881, p. 581.

Loc.: Honshiu (Nikko, Yamato); Siberia.

Time of appearance: June, July, August.

*11. *Macalla scoparialis*.

Macalla scoparialis Wileman, Trans. Ent. Soc. Lond. 1911, p. 365.

Loc.: Honshiu (Nikko).

Time of appearance: August.

Genus LOCASTRA.

Locastra Walker; Hampson, Trans. Ent. Soc. Lond. 1896, p. 469.

12. *Locastra muscosalis*.

(Pl. XXI, figs. 8, 9, 19.)

Taurica muscosalis Walker, Cat. XXXIV. p. 1269 (1865).

Locastra cristalis Hampson, Ill. Typ. Het. IX. p. 157, pl. 172. f. 3. (1893).

Locastra muscosalis Hampson, Moths Ind. IV. p. 119 (1896).

Loc. : Kiushiu (Nagasaki) ; Honshiu (Tokyo, Kii) ; China ; India ; Ceylon ; Rangoon.

Time of appearance : June, July.

Genus STERICTA.

Stericta Lederer ; Hampson, Trans. Ent. Soc. Lond. 1898, p. 470.

13. *Stericta haraldusalis*.

Stericta (?) *haraldusalis* Walker, Cat. XVI. p. 160 (1858).

Craneophora ficki Cristoph, Bull. Mosc. LVI (1). p. 2 (1881).

Scoparia variegata Moore, Lep. Atk. p. 203, pl. 7. f. 4 (1887).

Blenopholis striata Butler, Ill. Typ. Het. VII. p. 90, pl. 134. f. 3 (1889).

Stericta haraldusalis Hampson, Moths Ind. IV. p. 42 (1896).

Loc. : Honshiu (Tokyo, Kii) ; Corea ; India ; Siam ; Borneo ; Siberia ; China.

Time of appearance : May, June, July, September.

14. *Stericta olivacea*.

(Pl. XXI, fig. 11.)

Hyperbalunotis olibacca Warren, A. M. N. H. (6) VII. p. 433 (1891).

Orthaga olivacea Hampson, Trans. Ent. Soc. Lond. 1896, p. 476.

Loc. : Kiushiu ; Honshiu (Kii, Tokyo) ; Loochoo ; China.

Time of appearance : June, July, August.

This species should be placed under the genus *Stericta*, since the palpi of this species are longer than those of *Orthaga*.

*15. *Stericta olivalis*.

Stericta olivalis Wileman, Trans. Ent. Soc. Lond. 1911, p. 365, pl. 31. f. 20.

Loc. : Honshiu (Yoshino) ; India ?

Time of appearance : August.

Genus ORTHAGA.

Orthaga Walker; Hampson, Trans. Ent. Soc. Lond. 1896, p. 475.

16. *Orthaga euadrusalis*.

(Pl. XXI, fig. 12.)

Orthaga euadrusalis Walker, Cat. XVI. p. 191 (1858).

Orthaga acotialis Walker, Cat. XXVII. p. 103 (1863).

Loc. : Honshiu (Tokyo, Kii, Yoshino); Shikoku (Iyo); India; Ceylon; Borneo; Java.

Time of appearance : June, July, August.

17. *Orthaga achatina*.

Glossina achatina Butler, Ill. Typ. Het. 11. p. 56, pl. 38. f. 10 (1878).

Orthaga achatina Hampson, Trans. Ent. Soc. Lond. 1896, p. 476.

Loc. : Honshiu (Tokyo, Yokohama, Kii, Nagahama, Tsuruga, Fushiki); Kiushiu.

Time of appearance : July, August.

***18. *Orthaga grisealis*.**

Orthaga grisealis Wileman, Trans. Ent. Soc. Lond. 1911, p. 366.

Loc. : Honshiu (Yokohama, Yoshino).

Time of appearance : July, October.

***19. *Orthaga onerata*.**

Bleptina onerata Butler, A. M. N. H. (5) IV. p. 447 (1879).

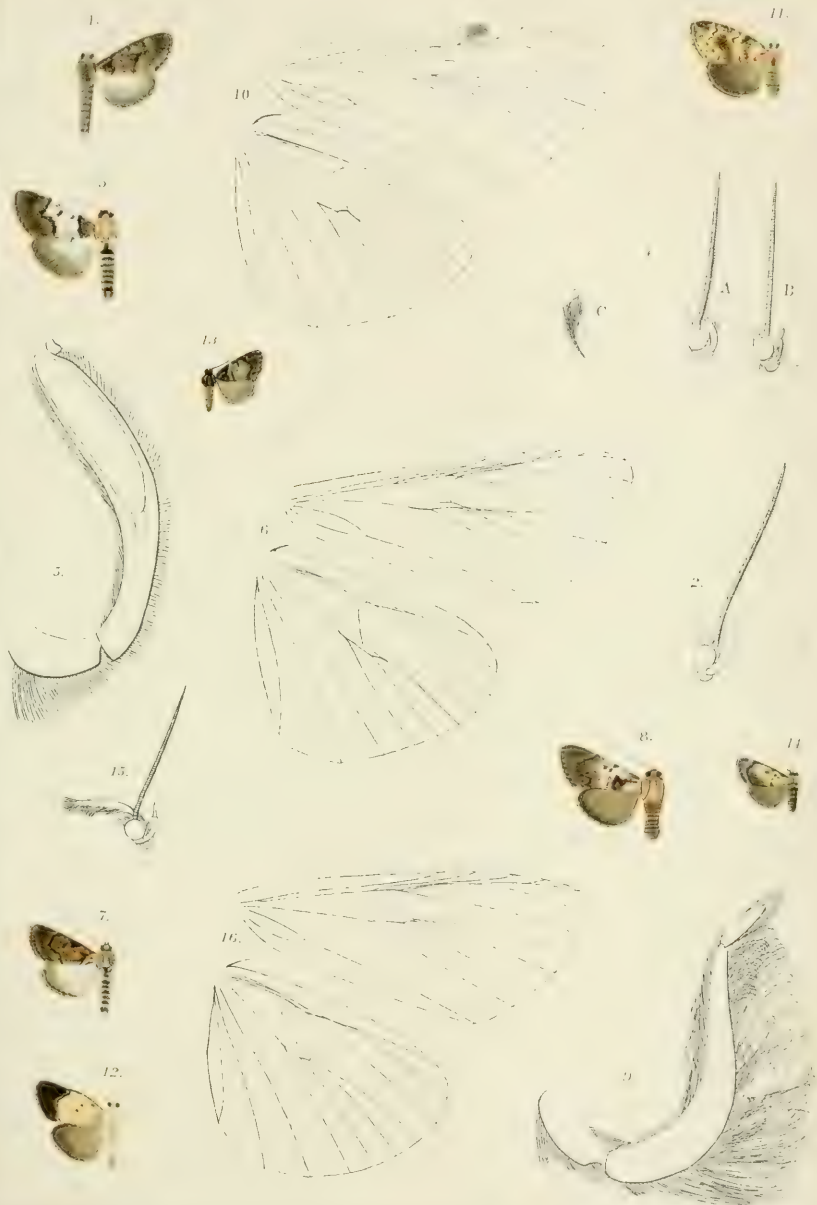
Orthaga onerata Hampson, Moths Ind. IV. p. 126 (1892).

Loc. : Honshiu (Yokohama, Nagahama); India; Borneo.

Time of appearance : July.

EXPLANATION OF PLATE XXI.

- Fig. 1. *Macalla moncusalis*, ♂. ×1.
Fig. 2. Head of ditto.
Fig. 3. *Macalla amica*, ♂. ×1.
Fig. 4. a, head of *Macalla amica*, ♂; b, ditto, ♀; c, palpus of ditto, ♂,
containing a brush-like maxillary palpus.
Fig. 5. Palpus of ditto, ♂. ×23.
Fig. 6. Wings of ditto, ♂. ×4.
Fig. 7. *Macalla inimica*, ♂. ×1.
Fig. 8. *Locastra muscosalis*, ♂, ×1.
Fig. 9. Palpus of ditto, ♂. ×23.
Fig. 10. Wings of ditto, ♂. ×4.
Fig. 11. *Stericta olivacea*, ♀. ×1.
Fig. 12. *Orthaga euadrusalis*, ♂. ×1.
Fig. 13. *Lepidogma japonica*, ♂. ×1.
Fig. 14. *Lepidogma kitensis*, ♂. ×1.
Fig. 15. Head of ditto, ♂.
Fig. 16. Wings of ditto, ♂. ×6.
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A Revision of the Notodontidæ* of Japan, Korea and Formosa with Descriptions of 5 New Genera and 5 New Species.

By

Nobukatsu Marumo.

With Plates XXII-XXXIX and 43 Text-Figures.

Introduction.

In 1898-1899, LEECH stated in his work¹ 56 species in the family Notodontidæ, which are found in both Japan and Korea, but the three species—*Stenoloba jankowskii* Oberthür, *Gelastocera erusta* Butler, and *Platychasma virgo* Butler—are to be removed from the family and included in the family Noctuidæ.

In 1901, STAUDINGER² mentioned 34, and in 1905 MATSUMURA³ 53 species.

In 1909, MATSUMURA added the following 6 new species, but these are not new to science and considered as synonymous as follows:

<i>Pheosia octofusciata</i> Matsumura	=	<i>Drymonia lineata</i> Oberthür
<i>Notodonta Ishidæ</i> "	=	<i>Lophodonta graseri</i> Staudinger
<i>Notodonta Nitobei</i> "	=	<i>Lophodonta aliena</i> Staudinger
<i>Spatalia Okamotois</i> "	=	<i>Spatalia dives</i> Oberthür
<i>Drymonia discoidalis</i> "	=	<i>Drymonia trimacula dodoniles</i> Staudinger
<i>Zangra ziozankeana</i> "	=	<i>Densitas permagnum</i> Butler

In 1910-1916, WILEMAN recognized the following 26 species together with the many unrecorded species from Japan and Formosa:

*The family name *Notodontidæ* should be amended to *Ceruidæ*.

1. LEECH: Lepidoptera Heterocera from N. China, Japan and Korea (Trans. Ent. Soc. Lond. 1898-1899).

2. STAUDINGER: Catalog der Lepidopteren des palaearctischen Faunengebietes. Berlin, 1901.

3. MATSUMURA: Catalogus Insectorum Japonicum. I. Tokyo, 1905.

<i>Phalera obscura.</i>	<i>Tarsolepis taiwan.</i>
<i>Stauropus viridipicta.</i>	<i>Stauropus bidentatus.</i>
<i>Stauropus nigribasalis.</i>	<i>Stauropus pulverulenta.</i>
<i>Stauropus confusa.</i>	<i>Fentonius variegata.</i>
<i>Fentonius nipponica.</i>	<i>Fentonius nigrofuscata.</i>
<i>Spatulia jaczansis.</i>	<i>Notodonta rothschildi.</i>
<i>Notodonta basilinea.</i>	<i>Notodonta lativitta.</i>
<i>Notodonta griseotincta.</i>	<i>Notodonta furva.</i>
<i>Notodonta? basinotata.</i>	<i>Liparopsis formosana.</i>
<i>Ochrostigma japonica.</i>	<i>Pydna kanshireiensis.</i>
<i>Pydna albifusa.</i>	<i>Pydna virgata.</i>
<i>Pydna sordida.</i>	<i>Pydna nelmulosa.</i>
<i>Pydna inconspicua.</i>	<i>Glyphisia japonica</i>

In 1912, GRÜNBERG described a new species, *Drymonia eximia*, and a new aberrant form, *Hypodonta pulcherrima stigmatica*, but to my consideration the former should be identical with *Fentonius nipponica* Wileman. At the same time he also founded a new genus *Gangaridopsis* for the reception of *Gangarides citrina* which was described by WILEMAN as an Eupterotid.

In the same year PÜNGELER recognized a new species, *Ochrostigma ussuriensis*, from Ussuri District. This should also be considered as synonymous with *Stauropus bidentatus* Wileman.

In 1916, NAGANO published his valuable paper on the life histories of some Japanese Lepidoptera, which contains the three new genera—*Wilemanus*, *Disparia*, *Micromelalopha*—a new species—*Phalera minor*—and a new varietical form—*Drymonia? manleyi coreana*. The genus *Disparia* seems to me to be sunk to *Macrurocampa* Dyar.

In 1917, WILEMAN and SOUTH recognized the three new species—*Drymonia basalis*, *Stauropus oblitera*, *Tarsolepis japonica*—from Japan.

Although many new species have been described by the above mentioned authors, their investigations look in some cases to be unsatisfactory. Thus, *Asterosecopus atrovittatus* Bremer has hitherto been included by several authors under the genus *Microphalera*. But a slight examination of it proves that the genus *Microphalera* is not the good place and a new genus should be required for it. The genus *Fentonius* BUTLER has been erroneously characterized by HAMMON including several Indian and Japanese species, but most of them may possibly be referable to the genus *Macrurocampa* Dyar.

In the following lines, I will try to remark on the already known species of the Notodontidæ of Japan, Corea and Formosa as far as I examined.

In describing the generic character great stress is layed upon the palpus and the lobe of the foretibiæ.

The number of the species of the Notodontidæ already recorded from there are 110 belonging to 40 genera, to which I am able to add 5 new genera and 5 new species.

As the two species, *Notodonta trachitso* Oberthür and *Pygera timon* Hübner recorded by GRÜNBERG are doubtful to exist in Japan, are put out from our list.

Before giving some remarks in each known species and describing new genera and species, I will mention their geographical distribution.

Species \ Locality	Hokkaido	Honshû	Shikoku	Kyushû	Corea	Formosa	Other Localities
1. <i>Phalera assimilis</i> Brem. et Grey	x	x		x	x		China, Siberia
2. <i>P. minor</i> Nagano		x					
3. <i>P. fuscescens</i> Butl.		x		x			
4. <i>P. flavescens</i> Brem.	x	x		x	x		China, Siberia
5. <i>P. combusta</i> Wlk.						x	China, India etc.
6. <i>P. obscura</i> Wileman						x	
7. <i>P. flavimacula</i> Wileman						x	
8. <i>Tarsolepis sommeri</i> Hübner		x		x			China, India etc.
9. <i>T. japonica</i> Wileman et South		x					
10. <i>T. taiwana</i> Wileman						x	
11. <i>Spatalia dives</i> Oberth.	x	x					Siberia
12. <i>S. doerriesi</i> Graes.	x	x	x				Siberia
13. <i>S. jezoensis</i> Wileman	x						
14. <i>S. plusiotis</i> Oberth.		x			x		China
15. <i>S. ornata</i> Oberth.		x	x				China
16. <i>S. cinnamomea</i> Leech		x		x			
17. <i>Cnethodonta grisescens</i> Staud.	x	x					Siberia
18. <i>Stauropus fagi</i> Linn.	x	x					China, Siberia, Europe

Species	Localities	Hokkaido	Honshu	Shikoku	Kyushu	Corea	Formosa	Other Localities
19. <i>Stauropus basalis</i> Moore		x	x		x			China, Siberia
20. <i>S. viridipictus</i> Wileman							x	
21. <i>S. nigrobasalis</i> Wileman							x	
22. <i>S. pulverulentus</i> Wileman							x	
23. <i>S. confusus</i> Wileman							x	
24. <i>S. oblitterata</i> Wileman et South							x	
25. <i>Hoplites milhauseri</i> Fabr.		x	x					China, Siberia
26. <i>Microhoplites circumscripta</i> Butl.		x	x					Europe
27. <i>Urodonta viridimixta</i> Brem.		x	x					Siberia
28. <i>U. uranicki</i> Oberth.		x	x					Siberia
29. <i>U. arcuata</i> Alph.			x					Siberia
30. <i>Lophocosma atriplaga</i> Staud.		x	x			x		Siberia
31. <i>Microphalera grisea</i> Butl.		x	x					Siberia
32. <i>Lophopteryx capucina</i> Linn.		x	x	x		x		Siberia, Europe
33. <i>L. saturata</i> Wlk.		x	x					India, Siberia
34. <i>L. ladislai</i> Oberth.		x	x					Siberia
35. <i>L. admirabilis</i> Staud.		x	x					Siberia
36. <i>L. velutina</i> Oberth.		x	x					Siberia
37. <i>Lophontesia cuculus</i> Staud.		x	x					Siberia
38. <i>L. pygmaea</i> Butl.		x	x					
39. <i>Allocladon leucodera</i> Staud.		x	x					Siberia, India
40. <i>A. gigantea</i> Butl.		x	x					Siberia?
41. <i>Yasawaka japonica</i> n. sp.			x					
42. <i>Cerura lanigera</i> Butl.			x	x		x		Siberia
43. <i>C. bifida</i> Hübn.		x		x?				China, Europe etc.
44. <i>C. furcula</i> Clerck						x		China, Siberia, Europe, etc.
45. <i>C. liturata</i> Wlk.							x	China, India etc.
46. <i>C. erminea</i> Esp.						x		China, Siberia, Europe
47. <i>C. vinula</i> Linn.		x	x			x	x	Siberia, Europe
48. <i>Macrurocampa sigmata</i> Butl.		x	x		x			China

Species	Localities	Hokkaido	Honshû	Shikoku	Kiushû	Corea	Formosa	Other Localities
49. <i>Macrurocamptis delia</i> Leech			x					
50. <i>M. nipponica</i> Wileman			x					
51. <i>M. variegata</i> Wileman			x				x	
52. <i>Macrurocamptis</i> ? <i>nigrofasciata</i> Wileman							x	
53. <i>Uropygia meticulodina</i> Oberth.	x	x						Siberia
54. <i>Fentonia ocypte</i> Brem.			x		x			Siberia, India
55. <i>Nerice davidi</i> Oberth.	x	x						China, Siberia
56. <i>N. bipartita</i> Butl.	x	x	x					China
57. <i>Brachionychooides atrovittatum</i> Brem.	x	x						Siberia
58. <i>Hyperaschra biloba</i> Oberth.	x	x						Siberia
59. <i>Il. basilinea</i> Wileman			x					
60. <i>Il. taiciana</i> n. sp.							x	
61. <i>Il. tenebrosa</i> Moore			x					India
62. <i>Notodonta dembowskii</i> Oberth.	x	x						Siberia
63. <i>N. stigmatica</i> Wileman	x	x?						
64. <i>N. tritoplus</i> Esp.	x							China, Siberia
65. <i>N. cinerea</i> Butl.	x	x						
66. <i>N. griseolincta</i> Wileman							x	
67. <i>N. furva</i> Wileman							x	
68. <i>N.? basinotata</i> Wileman							x	
69. <i>Ilupodonta pulcherrima</i> Moore	x	x						India
70. <i>Il. obsoleta</i> n. sp.	x							
71. <i>Pheosia dictyoides</i> Esp.			x					Siberia, Europe
72. <i>Leucodonta bicoloria</i> Schiff.			x					Siberia, Europe
73. <i>Wilemanus bidentatus</i> Wileman			x			x		Siberia
74. <i>Lophodonta graseri</i> Staud.	x	x						Siberia
75. <i>L. aliena</i> Staud.			x					Siberia
76. <i>L. lativitta</i> Wileman			x					
77. <i>L. monetaria</i> Oberth.	x	x						Siberia
78. <i>Euhampsonia cristata</i> Butl.	x	x						China, Siberia

Species	Localities						Other Localities
	Hokkaido	Honshu	Shikoku	Kyushu	Coro	Formosa	
79. <i>Eulampsonia splendida</i> Oberth.	x	x					China, Siberia
80. <i>Gangaridopsis citrina</i> Wileman		x					
81. <i>Himeropteryx miraculosa</i> Staud.	x			x			Siberia
82. <i>Pygopteryx suava</i> Staud.		x					Siberia
83. <i>Melalopha anastomosis</i> Linn.	x	x			x		China, Siberia, Europe, India etc.
84. <i>M. anachoreta</i> Fabr.	x	x	x		x		China, Siberia, Europe, India etc.
85. <i>M. curtuloides</i> Ersch.							Siberia
86. <i>Egonocia cyanea</i> Leech		x					
87. <i>E. formosana</i> n. sp.						x	
88. <i>E. wachiensis</i> n. sp.		x					
89. <i>E. fasciata</i> Moore		x					China, India
90. <i>E. perdit</i> Moore		x					China, India
91. <i>Drymonia linearis</i> Oberth.	x	x					Siberia
92. <i>D. trimacula</i> Esp.		x					Siberia, Europe
93. <i>D. basalis</i> Wileman et South		x					
94. <i>D. chaonia</i> Hübner		x					Europe
95. <i>Liparopsis formosana</i> Wileman						x	
96. <i>Oclerostigma japonica</i> Wileman		x					
97. <i>O. manleyi</i> Leech		x			x		
98. <i>O. punctatella</i> Motsch.	x	x					
99. <i>Philophora phanigera</i> Esp.	x	x					Siberia, Europe
100. <i>Ramesa tosta</i> Wlk.		x					India
101. <i>R. pallida</i> Batl.	x	x	x				China, India
102. <i>R. sordida</i> Wileman						x	
103. <i>R. nebulosa</i> Wileman						x	
104. <i>R. kanshireiensis</i> Wileman						x	
105. <i>R. straminea</i> Moore	x	x		x	x		China
106. <i>R. southerlandii</i> Holland		x					
107. <i>R. inconspicua</i> Wileman						x	
108. <i>R. plumosa</i> Leech		x					

Species	Localities	Hokkaido	Honshu	Shikoku	Kyushu	Corea	Formosa	Other Localities
109. <i>Ramesa virgata</i> Wileman							x	
110. <i>R. albifusa</i> Wileman							x	
111. <i>Pterostoma sinicum</i> Moore		x	x					China, Siberia
112. <i>Glusphisia japonica</i> Wileman		x	x					
113. <i>Micrometolopha troglodyta</i> Græs.			x					Siberia
114. <i>Gonoclostera timonides</i> Brem.		x	x	x				China, Siberia
115. <i>Densitas permagnum</i> Butl.		x	x					

I must express my hearty thanks to Prof. SASAKI who has permitted me to examine his original figures.

To Dr. MIYAKE whom I am deeply indebted for permission of examination of his valuable collections and for assistance of his kind advices I am also under special obligation.

Thanks are also due to Messrs. YANO, NAGANO, MITSUHASHI, YAMADA, HIRAYAMA and TAKAMUKU who have aided me with their collections or figures.

(In the following lines the species not examined by me are marked with *.)

Family NOTODONTIDÆ.

Proboscis usually more or less aborted in many genera, but sometimes fully developed as in *Pentonia*; palpi usually moderate, rarely abnormally developed as in *Pterostoma*; ocelli absent in most genera, rarely present; eyes usually naked, sometimes hairy; antennæ variable. Legs usually hairy; foretibiae with the lobe usually well developed; hindtibiae usually with two pairs of spurs, sometimes with only a pair. Wings almost always fully developed in both sexes. Forewing with 1a connected with 1b near the base to form a fork, but rarely 1a not connected with 1b; 1c absent; 5 from middle or from above middle of discocellulars, rarely from just below the upper angle of cell or rarely entirely absent. Hindwing with vein 1c absent; 5 from middle or from above middle of discocellulars or sometimes absent;

6 and 7 usually stalked, rarely both from the upper angle of cell; 8 free from the base, running close along the upper margin of cell to beyond middle and usually connected with it by a short bar or touching it. Frenulum usually present, rarely absent.

Larvæ with 14 or 16 feet. Head rather large. Body smooth or hairy and sometimes humped or tuberculated; with secondary setæ on the side of prolegs; anal legs sometimes prolonged into filaments.

"The pupæ of this family never show more than a small triangular or polygonal proximal portion of the labial palpi, and maxillary palpi are never present. The femora of the prothoracic legs are never exposed. The epicranial suture is present in the genera *Apatelodes* and *Melalopha*. The maxillæ never reach the caudal margin of the wings. The antennæ are always widest at their proximal ends, and there the width exceeds the greatest width of the prothoracic legs. Each antenna tapers gradually to a pointed tip and the tips often lie adjacent on the meson caudad of the other appendages. The metathoracic legs are seldom visible. The mesothoracic leg never reaches cephalad to the eye-pieces. The abdomen is always punctate and in most species the punctures are large. A cremaster is usually present and there are various types" (Mosher).

Many prominents resemble in general appearance to the Lymantrids and Noctuids, but they are easily distinguishable one from the other chiefly by the venation¹. In the venation they also resemble to the Geometrids, but readily distinguished from the latter by the basal part of vein 8 of the hindwing not making a prominent bent.

Although the venation is, as in the other families, one of the important characters in the classification of this family, it varies considerably even within the limits of the same species. For instance, the vein 6 of the forewing of *Phalera (Dinara) combusta* arises in some specimens from beyond upper angle of cell, but in others it arises from the upper angle of cell. The vein 10 is also subject to an individual variation in some species. The vein 8 of the hindwing which is used by HAMPSON (Catalogue of Lepidoptera Phalaenæ in the British Museum, Vol. I) in separating this family from the Geometridæ

1. In an Indian genus *Cyphanta* Hampson the vein 5 of the forewing arises from lower angle of cell.

is usually connected with the upper margin of cell by a short bar or touching it in a point. It is not, however, so in all cases. It therefore, requires much caution in using the venation as a generic character.

KEY TO THE GENERA.

- A. Forewing without tuft of scales on inner margin.
- a. Hindwing with vein 5 present.
- a¹. Forewing without areole.
- a². Hindwing with veins 7 and 8 bent upwards to the costa. *Liparopsis*.
- b². Hindwing with veins 7 and 8 not bent upwards to the costa.
- a³. Hindtibiae with one pair of spurs.
- a⁴. Forewing with vein 6 from upper angle of call or shortly stalked with 7, 8, 9 and 10; 10 from beyond 7.
- a⁵. Frenulum present.
- a⁶. Eyes naked.
- a⁷. Abdomen without dorsal series of crests *Cnethodonta*.
- b⁷. Abdomen with dorsal series of crests. *Stauropus*.
- b⁸. Eyes sparsely hairy. *Stilaphis*.
- b⁵. Frenulum absent. *Dorsitas*.
- a⁴. Forewing with vein 6 from far beyond the upper angle of cell; vein 10 from before 7.
- a⁵. Forewing with termen not strongly oblique; hindwing with veins 6 and 7 stalked to beyond middle. ... *Microhoplitis*.
- b⁵. Forewing with termen strongly oblique; hindwing with veins 6 and 7 not stalked to beyond middle. *Hoplites*.
- b³. Hindtibiae with two pairs of spurs.
- a⁴. Forewing with vein 10 from beyond vein 7.
- a⁵. Abdomen with dorsal series of crests. *Stauropus*.
- b⁵. Abdomen without dorsal series of crests.
- a⁶. Palpi not fringed with long woolly hair; forewing with the subcostal vein hairy on underside. ... *Egonocia*.
- b⁶. Palpi fringed with long woolly hair; forewing with the subcostal vein not hairy on underside ... *Drymonia*.
- b⁴. Forewing with vein 10 from before 7.
- a⁵. Palpi with 3rd joint little shorter than 2nd. *Microphalera*.
- b⁵. Palpi with 3rd joint much shorter than 2nd.
- a⁶. Forewing with termen more or less rounded, hind angle not sharply defined. *Ilyodontia*.
- b⁶. Forewing with termen oblique; hind angle sharply defined.

- b². Thorax without high erect crest.
 a³. Forewing with vein 10 from before vein 7.
 a⁴. Hindtibiae with two pairs of spurs. *Lophontosiæ*.
 b⁴. Hindtibiae with one pair of spurs. *Ptilophora*.
 b³. Forewing with vein 10 from beyond 7.
 a⁴. Eyes hairy. *Notodonta*.
 b⁴. Eyes not hairy.
 a⁵. Foretarsi thickly haired. *Phæasia*.
 b⁵. Foretarsi not thickly haired.
 a⁶. Forewing with inner margin much convexed.
 a⁷. Palpi woolly haired; forewing rather
 short and broad *Yuzawana*.
 b⁷. Palpi not woolly haired; forewing
 rather long. *Lophodonta*.
 b⁶. Forewing with inner margin slightly
 convexed. *Ochrostigma*.
 b. Forewing with an areole.
 a¹. Thorax with a high erect crest. *Lophopteryx*.
 b¹. Thorax without high erect crest.
 a². Palpi abnormally long. *Pterostoma*.
 b². Palpi normal.
 a³. Forewing with a tuft of scales at middle of inner margin
 and at hind angle. *Spatalia*.
 b³. Forewing with a tuft of scales at middle of inner margin.
 a⁴. Forewing with vein 10 from before 7. *Leurodonta*.
 b⁴. Forewing with vein 10 from beyond 7.
 a⁵. Forewing rather broad with termen more or less
 crenulate. *Himeropteryx*.
 b⁵. Forewing long with termen not crenulate. *Hyperæschra*.

Genus *Phalera* Hübner (1816).

Dinara Walker (1855).

Anticyra Walker (1855).

Palpi short, porrect and thickly scaled, the 3rd joint short; antennæ in male fasciculate or pectinate, in female ciliated or fasciculate?; proboscis present; eyes naked. Legs hairy, foretibiae with the lobe not reaching beyond the end, hindtibiae with two pairs of spurs.



Text.-fig. 1. Foretibia of *Phalera flavescens*, ♂. ×23.

Forewing elongate, without tuft

of scales on inner margin; vein 5 from middle or from above middle of discocellulus; 6, 7 and 8 stalked; 9 and 10 stalked, 9 or 9 and 10 anastomosing with 7 and 8 to form an areole. Hindwing with vein 5 from above middle of discocellulus; 6 and 7 shortly stalked; 8 running close along upper margin of cell, sometimes touching it.

Larvæ cylindrical, without humps, hairy; 16 feet. Pupate under the ground.

Pupæ with the cremaster provided with several spines.

Geographical distribution. Palaearctic and Oriental.

Sect. I. Antennæ in male fasciculate.

A. Palpi with 2nd joint slightly longer than 1st.

1. *Phalera assimilis*.

(Pl. XXII, fig. 3; Pl. XXIV, figs. 3, 4, 10; Pl. XXVIII, fig. 2;
Pl. XXXII, fig. 5.)

Pygara assimilis Bremer et Grey, Motschulsky, Etud. Ent. I. p. 30 (1852); Schmett. nörd. China. p. 16 (1853); KIRBY, Cat. Lep. Het. p. 577 (1892); ALPHÉRAKY, Rom. Mém. IX. p. 156, pl. 2. fig. 4 (1897); LEECH, Trans. Ent. Soc. Lond. 1898, p. 300; STAUDINGER, Cat. Lep. pal. p. 112 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 312, pl. 47c (1912); NAGANO, Bull. Nawa Ent. Lab. I. p. 4, pl. 2. figs. 17-21, pl. 9. fig. 13, larva (1916).

Phalera ningpoana Felder, Wien Ent. Mon. VI. p. 37 (1862).

Local distribution. Tokyo, Gifu, Kaga, Kiushiu, Hokkaido, Corea.

General distribution. Japan, Siberia, China, Corea.

Time of appearance. June—August.

In the male the apex of forewing is usually more acute than in the female.

2. *Phalera minor*.

Phalera minor Nagano, Bull. Nawa Ent. Lab. I. p. 7, pl. 2. figs. 1-5, pl. 9. fig. 22, larva (1916).

Local distribution. Gifu, Okayama.

Habitat. Japan.

Time of appearance. August.

3. *Phalera fuscescens*.

Phalera fuscescens Butler, Trans. Ent. Soc. Lond. 1881, p. 597; LEECH, Trans. Ent. Soc. Lond. 1898, p. 298; NAGANO, Bull. Nawa Ent. Lab. I. p. 6, pl. 2. figs. 6-16, pl. 9. fig. 18, larva (1916).

Phalera assimilis var? *fuscescens* Staudinger, Cat. Lep. pal. p. 112 (1901).

Local distribution. Tokyo, Gifu, Kiushiu.

Habitat. Japan.

Time of appearance. August.

B. Palpi with 2nd joint shorter than 1st.

4. *Phalera flavescens*.

(Pl. XXIV, figs. 1, 2; Pl. XXIX, fig. 3, Pl. XXXI, fig. 8; Text-fig. 1.)

Phalera flavescens Bremer, Schmiett. nörd. China. p. 14 (1853); STAUDINGER, Rom. Mém. VI. p. 368 (1892); Cat. Lep. pal. p. 111 (1901); LEECH, Trans. Ent. Soc. Lond. 1898, p. 299; GRÜNBERG, Seitz, Macrolep. II. p. 312, pl. 47e (1912); NAGANO, Bull. Nawa Ent. Lab. I. p. 8, pl. 2. figs. 22-27, pl. 9. fig. 25, larva (1916).

Trisura andreas Oberthür, Etud. Ent. V. p. 38, pl. 5. fig. 4 (1880).

Local distribution. Usuki (Kiushiu), Tokyo, Gifu, Hokkaido, Corea.

General distribution. Japan, Corea, China, Siberia.

Time of appearance. August.

Sect. II. Antennæ of male pectinate.

5. *Phalera combusta*.

(Pl. XXXIV, fig. 7.)

Antieyra combusta Walker, Cat. V. p. 1092 (1855); Hampson, Moths Ind. I. 145 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 302; GRÜNBERG, Seitz, Macrolep. II. p. 315, pl. 47e (1912).

Dinara lineolata Walker, Cat. VII. p. 1700 (1855).

Local distribution. Formosa.

General distribution. Formosa, China, India, Phillipines, Africa.

Time of appearance. April.

In one specimen examined by me vein 10 of forewing is not fully developed.

The following two species are unknown to me.

*6. *Phalera obscura*.

Phalera obscura Wileman, Entom. 1910, p. 138.

Local distribution. Kanshirei.

Habitat. Formosa.

Time of appearance. April.

*7. *Phalera flavimacula*.

Phalera flavimacula Wileman, Entom. 1912, p. 259.

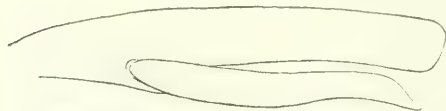
Local distribution. Arizan.

Habitat. Formosa.

Time of appearance. Unknown.

Genus *Tarsolepis* Butler (1872).

Palpi porrect and hairy, 3rd joint long; antennæ in male pectinate to beyond middle or "simple" (Grünberg) with a dense tuft at base; proboscis developed; eyes naked. Foretibiæ with the lobe extending almost to the



Text-fig. 2. Foretibia of *Tarsolepis sommeri*, ♂. ×23.

end, hindtibiæ with a pair of spurs. Abdomen long with a dense anal tuft of spatulate scales; first abdominal segment of male with a tuft of long

hair on each side. Forewing long with the apex acute; termen more or less crenulate; inner margin without tuft of scales; vein 5 from middle of discocellulars; 6, 7 and 8 stalked; 9 and 10 also stalked, 9 anastomosing with 7 and 8 or 8 only to form a long narrow areole. Hindwing with vein 5 from above middle of discocellulars; veins 6 and 7 stalked; 8 running close along upper margin of cell and connected with it by a short bar; traces of forked veinlets in the cell of both wings.

According to HAMPSON (Moths Ind. I. p. 127), vein 6 of the forewing of *T. sommeri* arises from angle of cell, but in the Japanese specimens of the same species it stalks with 7 and 8; in his figure of the venation of the forewing both veins 9 and 10 anastomose with 8 and a short areole is formed, while in the four Japanese specimens examined by me vein 9 only anastomoses with 8, and vein 10 arises from the areole which is long and narrow. More over he has erroneously characterized the genus stating "hind femur with a tuft of long hair from near the extremity." As shown in fig. 8b, pl. XI, the tuft is situated on the lateral side of the 1st abdominal segment of male.

Geographical distribution. Palearctic and Oriental.

8. *Tarsolepis sommeri*.

(Pl. XXII, fig. 14; Pl. XXVII, fig. 10; Pl. XXVIII, fig. 1;
Pl. XXXI, fig. 9; Text-fig. 2.)

Crino sommeri Hübner, Samml. Ex. Schmett. Noc. Gen. IV. figs. 1, 2 (1824?);

KIRBY, Cat. Lep. Het. p. 616 (1892); LEECH, Trans. Ent. Soc. Lond. 1898. p. 297; GRÜNBERG, Seitz, Macrolep. II. p. 284, pl. 48h (1912).

Tarsolepis remicauda Butler, A.M.N.H. (4) X. p. 125, pl. 8.

Local distribution. Tokyo, Kyoto, Shinano, Kii, Kiushiu.

General distribution. Japan, China, India, Borneo, Phillipines.

Time of appearance. July.

Hindtibiae with only a pair of spurs and not two pairs as Grünberg has stated. The costal margin of the forewing in female is more strongly arched than in male.

*9. *Tarsolepis japonica*.

Tarsolepis japonica Wileman et South, Entom. 1917, p. 29.

Local distribution. Tokyo, Miyanoshta.

Habitat. Japan.

Time of appearance. July, August.

*10. *Tarsolepis taiwana*.

Tarsolepis taiwana Wileman, Entom. 1910, p. 138.

Local distribution. Rantaizan.

Habitat. Formosa.

Time of appearance. May.

Genus *Spatalia* Hübner (1816).

Rosama Walker (1855).

Palpi obliquely upturned, 3rd joint short and rounded or long and pointed; antennæ in male pectinate or serrate and fasciculate; proboscis present; eyes naked. Legs more or less smooth or hairy; foretibiæ with the lobe extending to the end, hindtibiæ with two pairs of spurs. Abdomen in male usually long, with lateral tufts of hair and two terminal tufts well developed. Forewing with inner margin more or less excised; a large tuft of scales at middle of inner margin and at anal



Text-fig. 3. Foretibia of *S. doerriesi*, ♂. ×23.

angle; vein 5 from middle of discocellulars; 6 from just below upper angle of cell or stalked with 7 and 9; 9 and 10 stalked; 9 or 9 and 10 anastomosing with 8 or both 7 and 8 to form a long or short areole; the stalk of 9 and 10 sometimes coincides with the stalk of 7 and 8 or of 6, 7 and 8 to form no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larva "slender, smooth and naked, with 16 feet, on abdominal segment 1 a strong transverse swelling which bears a transverse row of 4 small tubercles, abdominal segment 8 likewise 2 obtuse tubercles" (GRÜNBERG).

Pupæ "in a slight web, anal end with a few small hooks" (GRÜNBERG).

Geographical distribution. Palearctic and Oriental.

In the Fauna of British India, Moths. Vol. I, HAMPTON has stated veins "9 and 10 anastomosing with 8 to form the areole," while SPULER in his work, Schmetterling Europas. Band I, "ohne Anhangszelle." GRÜNBERG seems to me to follow HAMPTON's characterization. The result of my examination on the genus *Spatalia* is as follows:

1. *Spatalia* (*Rosama*) *cinnamomea* Leech. Forewing broad; the areole present, long and narrow; vein 7 from the areole; antennæ pectinate to near the tip, the branches long and slender; tibial spurs long.

2. *Spatalia* (*Ptilodontis*) *ornata* Oberthür. Almost similar to the preceding species.

3. ? *Spatalia* (*Ptilodontis*) *plusiotis* Oberthür, ♂. Forewing narrower; the areole present, but very small or entirely absent; vein 7 anastomosing with the stalk of 7, 8 and 9, arising from beyond the areole, or it is stalked with 7, 8, 9 and 10 arising from before 10; antennæ of male pectinate to about two-thirds, the branches short and stiff.

4. *Spatalia doerriesi* Graeser, ♂. Forewing almost similar to the preceding; the areole entirely absent; vein 10 from beyond 7; antennæ pectinate to two-thirds, the branches very short and stiff (about half the length of those of the preceding species and it may be said by some one to be serrate); tibial spurs somewhat shorter than in the three preceding species.

5. *Spatalia dives* Oberthür, ♂. Forewing almost like the preceding species, but somewhat broader; the areole also entirely absent; vein 10 as in the preceding; antennæ not pectinate, but very minutely serrate and fasciculate.

Speaking of the silvery spot which is one of the characteristics of the genus, the first two have nothing of it in the female or sometimes very feebly developed in *ornata*; in the male of *cinnamomea* a golden yellow spot (instead of silvery) is present and in that of *ornata* a smaller silvery spot. Although I have no material to dispute of the female in the last three species, the male has more complicated silvery spot and the simplest of them seems to me to be *plusiotis*. The silvery spot of *dives* has very slightly golden lustre.

The fact that in *plusiotis* the areole is present or absent shows that *plusiotis* stands between the first two (*cinnamomea* and *ornata*) and the last two species (*doerriesi* and *dives*), and in the length of the branches of the male antennæ and in the arrangement of the silvery spot it also indicates the transition from the first two to the last two species.

Sect. I. Antennæ of male minutely serrate and fasciculate; forewing with the areole entirely absent; palpi with 3rd joint rather broad and obtuse.

11. *Spatalia dives*.

(Pl. XXIII, fig. 20; Pl. XXIV, fig. 9; Pl. XXVIII, fig. 9;

Pl. XXXIV, fig. 1.)

Spatalia dives Oberthür, Etud. Ent. X. p. 15, pl. 1. fig. 1 (1884); STAUDINGER,

Cat. Lep. pal. p. 110 (1910); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 297; GRÜNBERG, Seitz, Macrolep. II. p. 303, pl. 46f (1912).

Spatalia Okamotoi Matsumura, Zoku-senchiu-zukai. I. p. 82, pl. 11, fig. 22 (1909).

Local distribution. Honshiu, Hokkaido.

General distribution. Japan, Siberia.

Time of appearance. July, August.

Sect. 11. Antennae of male pectinate with the branches short and stiff; forewing with the areole present or absent; palpi with 3rd joint broad and obtuse.

12. *Spatalia doerriesi*.

(Pl. XXIV, figs. 8, 12; Pl. XXVIII, fig. 8; Pl. XXXII, fig. 5; Text-fig. 3.)

Spatalia doerriesi Græser, Berl. ent. Zeit. 1888, p. 141; STAUDINGER, Cat. Lep. pal. p. 109 (1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 297; GRÜNBERG, Seitz, Macrolep. II. p. 303, pl. 46e (1912),

Spatalia plusiotis (part) Staudinger (nec Oberthür), Rom. Mém. VI. p. 359 (1892).

Local distribution. Nikko, Kamikochi, Matsuyama, Hokkaido.

General distribution. Japan, Siberia.

Time of appearance. July.

*13. *Spatalia jezoensis*.

Spatalia jezoensis Wileman, Entom. 1916, p. 133.

Spatalia doerriesi (part) Wileman (nec Græser), Trans. Ent. Soc. Lond. 1911, p. 297.

Local distribution. Hokkaido (Tobetsu).

Habitat. Japan (Hokkaido).

Time of appearance. July.

14. ?*Spatalia plusiotis*.

(Pl. XXIII, fig. 19; Pl. XXIV, figs. 7, 13; Pl. XXVIII, fig. 6; Pl. XXXIV, fig. 2.)

Ptilodontis plusiotis Oberthür, Etud. Ent. V. p. 65, pl. 7, fig. 3 (1880); Kirby,

Cat. Lep. Het. p. 597 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 315; STAUDINGER, Cat. Lep. pal. p. 109 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 303, pl. 46e (1912).

?*Spatalia argentifera* Matsumura (nec Walker), Zoku-senchiu-zukai. I. p. 80, pl. 11. fig. 18 (1909).

Local distribution. Nikko, Oiwake, Shirahone, Yokohama, Corea.

General distribution. Japan, Corea, Siberia.

Time of appearance. July.

The specimen figured in Pl. XXIII differs from OBERTHÜR's figure, but I can not identify the specimen with other species.

Sect. III. Antennæ of male pectinate with the branches long; forewing with the areole present; palpi with 3rd joint slender and acute.

15. *Spatalia ornata*.

(Pl. XXVIII, fig. 5; Pl. XXXII, fig. 4.)

Psilodontis ornata Oberthür, Etud. Ent. X. p. 15, pl. 2. fig. 5 (1884); STAUDINGER, Rom. Mém. VI. p. 362 (1892); Cat. Lep. pal. p. 110 (1901); LEECH, Trans. Ent. Soc. Lond. 1898, p. 315; GRÜNBERG, Seitz, Macrolep. II. p. 304, pl. 46f (1912).

?*Rosama macrodonta* Butler, Cist. Ent. III. p. 127 (1885).

Local distribution. Shimoosa, Tokyo, Kaga, Yokohama, Shikoku,

General distribution. Japan, China.

Time of appearance. June, August.

The silvery spot at the base of the vein 2 of the forewing is much reduced in size or entirely absent in the female.

Although I have not examined the types of both *ornata* and *macrodonta* I can not find any specific distinction between them according to their original descriptions.

16. *Spatalia cinnamomea*.

(Pl. XXIII, fig. 18; Pl. XXIV, figs. 6, 11; Pl. XXVIII, fig. 4;
Pl. XXXII, fig. 3.)

Rosama cinnamomea Leech, Proc. Zool. Soc. Lond. 1888, p. 637. pl. 31.

fig. 11; Trans. Ent. Soc. Lond. 1898, p. 315; GRÜNBERG, Seitz, Macrolep. II. p. 304, pl. 46d (1912).

Local distribution. Tokyo, Kyoto, Ohoyama, Usuki, Nagasaki.

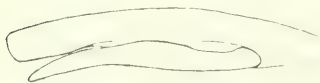
Habitat. Japan.

Time of appearance. June, August.

♂. Darker than female, but the most remarkable distinction between sexes is the presence of a golden-yellow triangular spot at the base of vein 2 in ♂.

Genus *Cnethodonta* Standinger (1887).

Palpi porrect or obliquely upturned, 3rd joint moderate and obtuse; proboscis vestigial; antennae in both sexes pectinate to the tip, the branches long in male, shorter in female; eyes naked. Legs hairy; foretibia with the



Text-fig. 4. Foretibia of *C. grisescens*, ♂. × 23.

lobe extending to just before the end, hindtibiae with only a pair of spurs. Forewing without tuft of scales on inner margin; vein 5 from about middle of discocellulars; 7, 8, 9 and

10 stalked; no areole. Hindwing with veins 3 and 4 from lower angle of cell, which is much produced and pointed; 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell and connected with it by a short bar at middle.

Early stages unknown.

Geographical distribution. Palearctic.

17. *Cnethodonta grisescens*.

(Pl. XXIII, fig. 3; Pl. XXVIII, fig. 7; Pl. XXXIV, fig. 5;

Text-fig. 4.)

Cnethodonta grisescens Standinger, Rom. Mém. III. p. 214, pl. 12. fig. 11 (1887); Cat. Lep. pal. p. 107 (1901); LEECH, Trans. Ent. Soc. Lond. 1898, p. 305; GRÜNBERG, Seitz, Macrolep. II. p. 290, pl. 45b (1912).

Dashyehira acronycta Oberthür, Etud. Ent. V. p. 35, pl. 5. fig. 8 (1880).

Local distribution. Tokyo, Kaga, Shinano, Hokkaido.

General distribution. Japan, Siberia.

Time of appearance. August.

I have taken in Kaga several male specimens which are recognized as an aberrant form of this species. In these specimens the subterminal series of specks of the forewing is so indistinct as it is hardly traceable and the size of moths (Expanse 39 mm.) is much smaller than that of the typical form.

Genus **Stauropus** Germer (1811).

Palpi short, porrect and hairy, 3rd joint minute; proboscis vestigial; antennæ pectinate to two-third in male, ciliated in female, or pectinate in both sexes, or fasciculate in male, simple in female" (Hampson); eyes naked. Legs hairy; foretibiæ with the lobe reaching the end, hindtibiæ with one or



Text-fig. 5. Foretibia of *S. fagi*, ♂. × 23.

two pairs of spurs. Abdomen with dorsal series of crests on basal segments. Forewing with the subcostal nervure hairy on underside; without

tuft of scales on inner margin; vein 5 from middle of discocellulars; 6 from upper angle of cell or stalked with 7, 8, 9 and 10; no areole. Hindwing with vein 5 from middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell and connected with it by a short bar at middle.

Larvæ. Head large, body slender with terminal two segments large; meso- and meta-thoracical legs as well as anal legs prolonged and slender; from 4th to 9th segments each with a pair of dorsal processes; head and body provided with short hair. When they rest they raise up both the anterior and posterior portions of the body so as to give a striking appearance. They spin a rough cocoon between leaves.

Pupæ "with pointed cremaster bearing two small bristles" (GRÜNBERG).

Geographical distribution. Palearctic and Oriental.

Sect. I. Antennæ in male pectinate, in female ciliated.

A. Hindtibiæ with one pair of spurs.

18. *Stauropus fagi*.

(Pl. XXVII, fig. 8; Pl. XXXIII, fig. 1; Text-fig. 5.)

Noctua fagi Linnaeus, Syst. Nat. I. p. 508 (1758); HÜBNER, Bomb. pl. 8. fig. 31; HAMPSON, Moths Ind. I. p. 149 (1892); LEECH, Trans. Ent. Soc.

Lond. 1898, p. 306; STAUDINGER, Cat. Lep. pal. p. 107 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 289, pl. 44g (1912).

Stauropus persimilis Butler, A.M.N.H. (5) IV. p. 353 (1879).

Local distribution. Tokyo, Oiwake, Yokohama, Hokkaido.

General distribution. Japan, China, Siberia, Europe.

Time of appearance. May, July, August.

B. Hindtibiae with two pairs of spurs.

19. *Stauropus basalis*.

(Pl. XXIII, fig. 2; Pl. XXVII, fig. 7; XXVIII, fig. 10;

Pl. XXXIII, fig. 2.)

Stauropus basalis Moore, A.M.N.H. (4) XX. p. 90 (1877); LEECH, Trans. Ent.

Soc. Lond. 1898, p. 306; STAUDINGER, Cat. Lep. pal. p. 107 (1901);

GRÜNBERG, Seitz, Macrolep. II. p. 290, pl. 44g (1912).

Harpyia taczanowskii Oberthür, Diagn. Lep. Askold. p. 11 (1879).

Stauropus fagi Matsumura (nec Linnaeus), Zoku-senchiu-zukai. I. p. 75, pl. 11, fig. 10 (1909).

Stauropus basalis ab. *niphonica* Grünberg, Seitz, Macrolep. II. p. 290, pl. 44g (1912).

Local distribution. Tokyo, Kaga, Yokohama, Usuki, Hokkaido.

General distribution. Japan, China, Siberia.

Time of appearance. May, August.

Larvæ feed on *Rubus parvifolius* (according to Prof. SASAKI).

The following 5 species are unknown to me.

*20. *Stauropus viridipictus*.

Stauropus viridipicta Wileman, Entom. 1910. p. 312.

Local distribution. Kanshirei.

Habitat. Formosa.

Time of appearance. May.

*21. *Stauropus nigribasalis*.

Stauropus nigribasalis Wileman, Entom. 1910. p. 289.

Local distribution. Rantaizan.

Habitat. Formosa.

Time of appearance. May.

*22. *Stauropus pulverlentus*.

Stauropus pulverlentus Wileman, Entom. 1910, p. 289.

Local distribution. Kanshirei.

Habitat. Formosa.

Time of appearance. April.

*23. *Stauropus confusus*.

Stauropus confusa Wileman, Entom. 1910, p. 389.

Local distribution. Rantaizan.

Habitat. Formosa.

Time of appearance. May.

*24. *Stauropus obliteratedus*.

Stauropus obliterateda Wileman, Entom. 1917, p. 29.

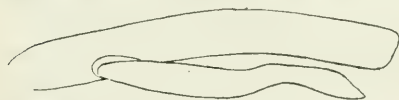
Local distribution. Oiwake.

Habitat. Japan.

Time of appearance. Unknown.

Genus **Hoplites**¹ Hübner (1816).

Palpi short, porrect and hairy, first two joints broad, third small and obtuse; antennæ pectinate to two-thirds in both sexes; proboscis vestigial; eyes naked. Legs hairy; foretibiæ with the lobe extending to the end, hindtibiæ



Text-fig. 6. Foretibia of *H. milhauseri*, ♂. ×23.

with a pair of spurs. Forewing elongate with termen strongly oblique and apex more or less acute; without tuft of scales on inner margin; vein 5 from

middle of discocellulars; 6, 7, 8, 9 and 10 stalked; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked to before middle; 8 running close along upper margin of cell.

1. *Cerura* Schrank (1802) has priority over *Hoplites* Hübner (1816).

Larvæ (according to European authors) almost naked with 14 feet; anal segment posteriorly truncate; segments 4-9 humped above, the first process largest; a large process on the 11th segment. They spin a compact cocoon attached to the trunk of the tree.

Pupæ "short and stout; a pointed tubercle on the vertex of head" (GRÜNBERG).

Geographical distribution. Palearctic.

25. *Hoplites milhauseri*.

(Pl. XXII, fig. 7; Pl. XXVII, figs. 11, 14; Pl. XXXVI, fig. 1;

Text-fig. 6.)

Bombyx milhauseri Fabricius, Syst. Ent. p. 577 (1775); KIRBY, Cat. Lep. Het. p. 595 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 309; STAUDINGER, Cat. Lep. pal. p. 107 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 292, pl. 45a (1912).

Bombyx terrifica Borkhausen, Eur. Schmett. III. p. 387 (1790); Hübner, Bomb. pl. 8. figs. 32, 33.

Hybocampa milhauseri var. *umbrosa* Staudinger, Rom. Mém. VI. p. 343 (1892).

Local distribution. Tokyo, Ohoyama, Yokohama, Hokkaido.

General distribution. Japan, China, Siberia, Europe.

Time of appearance. May.

Genus *Microhoplitis* n. gn.

Palpi minute, porrect, fringed with long woolly hair, 1st joint longest, 2nd and 3rd almost equal in length; proboscis vestigial; antennæ in male pectinate almost to apex; eyes naked. Legs hairy; foretibiae with the lobe



Text-fig. 7. Foretibia of *M. circumscripta*, ♂. ×23.

extending to the end, hindtibiae with a pair of spurs. Forewing with the termen strongly oblique and without tuft of scales on inner margin; vein 5 from middle of

discoecellulars; 6 from upper angle of cell; 7, 8, 9 and 10 stalked. Hindwing with vein 5 from above middle of discoecellulars; 6 and 7 stalked to beyond

middle; 8 running close along upper margin of cell. Abdomen with anal tuft more or less developed.

Early stages unknown.

Type. *M. circumscripta*.

Closely allied to the preceding genus *Hoplites*, but readily distinguishable from it by the longer stalk of veins 6 and 7 in hindwing.

Geographical distribution. Palearctic.

26. *Microhoplitis circumscripta*.

(Pl. XXII, fig. 8; Pl. XXVII, fig. 15; Pl. XXXIV, fig. 6;

Text-fig. 7.)

Drymonia circumscripta Butler, Cist. Ent. III. p. 125 (1885); LEECH, Trans.

Ent. Soc. Lond. 1898, p. 303; MATSUMURA, Zoku-senchiu-zukai. I. p. 81, pl. 11. fig. 25 (1909); GRÜNBERG, Seitz, Macrolep. II. p. 297 (1912).

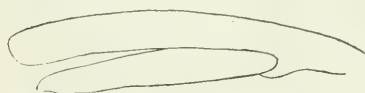
Local distribution. Kaga, Nikko, Hokkaido.

Habitat. Japan.

Time of appearance. August.

Genus *Urodonta* Staudinger (1887).

Palpi porrect, hardly reaching beyond the short sharp frontal tuft, moderately fringed with hair; antennæ in male pectinate to two-thirds, in female serrate and fasciculate or ciliated. Legs hairy; foretibiæ with the lobe hardly



Text-fig. 8. Foretibia of *U. viridimixta*, ♂. ×23.

reaching the end, hindtibiæ with two pairs of spurs. Forewing without tuft of scales on inner margin; vein 5 from about middle of discocellulars; 6, 7, 8, 9 and 10

stalked; no areole. Hindwing with vein 5 from just above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larva (*U. albinacula*) "likewise resembles those of the species of *Notodonta*; it is cylindrical and bears on the terminal segment a small conical tubercle whose tip is divided into 2 slight small warts" (GRÜNBERG).

According to GRÜNBERG, the palpi of the moths of this genus are moderately large; to my examination, however, the female of *U. viridimixta*

has the very minute palpi concealed beneath the hairs of the frons. The antennæ of the female of the same species are biserrate to two-thirds and those of *branicki* ciliated, while GRÜNBERG states "the antennæ pectinate to 3/4, the branches short in ♀, longer in ♂."

Geographical distribution. Palearctic.

27. *Urodonta viridimixta*.

(Pl. XXII, fig. 16; Pl. XXIV, fig. 5; Pl. XXVI, fig. 10;

Pl. XXXV, fig. 2; Text-fig. 8.)

Mischia viridimixta Bremer, Lep. Ost-Sib. p. 52, pl. 5. fig. 12 (1864);
STAUDINGER, Rom. Mém. III. p. 219 (1887); Cat. Lep. pal. p. 107
(1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 287; GRÜNBERG, Seitz,
Macrolep. II. p. 293, pl. 46c (1912).

Drymonia lichen Oberthür, Etud. Ent. V. p. 64, pl. 8. fig. 5 (1880).

Local distribution. Shimoosa, Sapporo, Tobetsu, Junsai Numa.

General distribution. Japan, Siberia.

Time of appearance. April, July, August.

28. *Urodonta branicki*.

Uropus branicki Oberthür, Etud. Ent. V. p. 60, pl. 6. fig. 6 (1880);

STAUDINGER, Rom. Mém. VI. p. 346 (1892); Cat. Lep. pal. p. 107
(1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 293; GRÜNBERG,
Seitz, Macrolep. II. p. 293, pl. 46c (1912).

Local distribution. Gifu, Tokyo, Junsai Numa.

General distribution. Japan, Siberia.

Time of appearance. April, July.

Allied to the preceding species, but differs from it in the female antennæ being ciliated instead of being biserrate.

*29. *Urodonta arcuata*.

Urodonta arcuata Alpheraky, Rom. Mém. IX. p. 154, pl. 11. fig. 9 (1897);

STAUDINGER, Cat. Lep. pal. p. 107 (1901); WILEMAN, Trans. Ent. Soc.
Lond. 1911, p. 293; GRÜNBERG, Seitz, Macrolep. II. p. 293, pl. 49a (1912).

Local distribution. Honshiu.

General distribution. Japan, Siberia.

Time of appearance. Unknown.

Genus **Lophocosma** Standinger (1887).

Palpi obliquely upturned to the centre of frons, first two joints hairy, 3rd rather long and somewhat pointed; antennæ in male pectinate to the tip, in female ciliated; eyes hairy; proboscis present. Thorax with a high erect crest. Legs hairy; foretibiae



Text-fig. 9. Foretibia of *L. atriplaga*, ♂. × 23.

with the lobe not reaching the end; hindtibiae with two pairs of spurs. Forewing without tuft of scales on inner

margin; vein 5 from middle of discocellulars; 6 from upper angle of cell; 7, 8 and 9 stalked; 10 from cell and anastomosing with 7, 8 and 9 to form a short areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Early stages unknown.

Geographical distribution. Palearctic.

30. ***Lophocosma atriplaga*.**

(Pl. XXII, fig. 12; Pl. XXVII, fig. 9; Pl. XXVIII, fig. 11;

Pl. XXXIII, fig. 3; Text-fig. 9.)

Notodonta (*Lophocosma*) *atriplaga* Standinger, Rom. Mém. III. p. 220, pl. 12. fig. 8 (1887); Cat. Lep. pal. p. 107 (1901); KIRBY, Cat. Lep. Het. p. 606 (1892); LEECH, Trans. Ent. Soc. Lond. 1893, p. 311; GRÜNBERG, Seitz, Macrolep. II. p. 294, pl. 46c (1912).

Local distribution. Tokyo, Hokkaido, Corea.

General distribution. Japan, Corea, Siberia.

Time of appearance. July.

Genus **Microphalera** Butler (1885).

Palpi short and porrect, three joints almost equal in length; proboscis feebly developed; antennæ in male pectinate to the tip; eyes naked. Legs

hairy; foretibiae with the lobe extending to three-fourths, hindtibiae with two pairs of spurs. Forewing with vein



Text-fig. 10. Foretibia of *M. grisea*, ♂. ×23.

5 from middle of discocellulars; 6 from upper angle of cell or stalked with 7, 8, 9 and 10; no areole; no tuft of scales on inner margin.

Hindwing with vein 5 from above middle of discocellulars which are oblique; 6 and 7 stalked; 8 running close along upper margin of cell.

Early stages unknown.

Geographical distribution. Palearctic.

31. *Microphalera grisea*.

(Pl. XXIII, fig. 12; Pl. XXVII, fig. 5; Pl. XXIX, fig. 10;

Pl. XXXIII, fig. 4; Text-fig. 10.)

Microphalera grisea Butler, Cist. Ent. III. p. 120 (1885); LEECH, Trans. Ent. Soc. Lond. 1898, p. 310; STAUDINGER, Cat. Lep. pal. p. 109 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 299 (1912).

Local distribution. Tokyo, Kyoto, Hakodate.

General distribution. Japan, Siberia.

Time of appearance. June.

Genus *Lophopteryx* Stephens (1828).

Palpi porrect, first two joint thickly scaled, 3rd small and more or less pointed; proboscis vestigial; antennae in male serrate and fasciculate or shortly pectinate, in female dentate and ciliated; eyes slightly hairy. Thorax



Text-fig. 11. Foretibia of *L. capucina*, ♂ from Europe. ×23.

with a high erect crest.

Legs hairy; foretibiae with the lobe extending to about

three-fourths, hindtibiae with two pairs of spurs. Forewing with termen slightly crenulate; a tuft of scales at middle of inner margin; vein 5 from middle of discocellulars; 6 from upper angle of cell or just above it; 7, 8 and 9 stalked; 10 from cell

and anastomosing with 7, 8 and 9 or 8 and 9 to form an areole. Hindwing with vein 5 from just above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ "cylindrical, with 16 feet, naked, only clothed with single long hairs or sparse tufts, on segment 8 of abdomen a single or double tubercle. When at rest the fore and hind parts are raised" (GRÜNBERG).

Pupæ "with or without spines at the anal end" (GRÜNBERG).

Geographical distribution. Palearctic, Oriental and Nearetic.

32. *Lophopteryx capucina*.

(Pl. XXIII, fig. 21; Pl. XXV, figs. 1, 9; Pl. XXXI, fig. 1;

Pl. XXXVII, fig. 2; Text-fig. 11.)

Bombyx capucina Linnaeus, Syst. Nat. I. p. 507, No. 55 (1758); KIRBY, Cat.

Lep. Het. p. 605 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 312.

Bombyx camelinæ Linnaeus, l.c. p. 507, No. 56.

Lophopteryx camellina Matsumura (nec LINNEUS), Cat. Ins. Jap. I. p. 37 (1905).

Bombyx giraffina Hübner, Bomb. figs. 277, 278 (1800?).

Local distribution. Nikko, Fujisan, Oiwake, Shirouma-dake, Kagu, Hakodate, Shikoku, Corea.

General distribution. Japan, Corea, Siberia, Europe.

Time of appearance. June.

The Japanese specimens may possibly be referable to the form *giraffina*.

33. *Lophopteryx saturata*.

(Pl. XXIII, fig. 22.)

Lophopteryx saturata Walker, Cat. XXXII. p. 415 (1865); Butler, Ill. Het.

Brit. Mus. VI. p. 25. pl. 107. fig. 1 (1886); HAMPSON, Moths Ind. I.

p. 166 (1892); STAUDINGER, Cat. Lep. pal. p. 110 (1901); WILEMAN, Trans.

Ent. Soc. Lond. 1911. p. 295; GRÜNBERG, Seitz, Macrolep. II. p. 307,

pl. 46h (1912).

Lophopteryx saturata var. *hagei* Græser, Berl. ent. Zeit. 1888, p. 141.

Local distribution. Karuizawa, Junsai Numa, Tobetsu, Hakodate, Sapporo.

General distribution. Japan, India, Siberia.

Time of appearance. July, August, September.

The form found in Japan may possibly be referable to *haegi*.

34. *Lophopteryx ladislai*.

(Pl. XXV, fig. 5 ; Pl. XXXI, fig. 2 ; Pl. XXXVI, fig. 8.)

Lophopteryx ladislai Oberthür, Etud. Ent. V. p. 66, pl. 2. fig. 3 (1880) ;

LEECH, Trans. Ent. Soc. Lond. 1898, p. 313 ; STAUDINGER, Cat. Lep. pal.

p. 110 (1901) ; GRÜNBERG, Seitz, Macrolep. II. p. 307, pl. 46h (1912).

Local distribution. Yokohama, Oiwake, Tokyo?, Hokkaido.

General distribution. Japan, Siberia.

Time of appearance. August.

35. *Lophopteryx admirabilis*.

(Pl. XXV, fig. 6 ; Pl. XXVI, fig. 9 ; Pl. XXXI, fig. 2.)

Lophopteryx admirabilis Staudinger, Rom. Mém. III. p. 224, pl. 12. fig. 9

(1887) ; Cat. Lep. pal. p. 110 (1901) ; Wileman, Trans. Ent. Soc. Lond.

1911, p. 296 ; Grünberg, Seitz, Macrolep. II. p. 307, pl. 46h (1912).

Local distribution. Nikko, Tobetsu.

General distribution. Japan, Siberia.

Time of appearance. August.

Antennæ in male shortly pectinate and the palpi with 1st joint rather long.

36. *Lophopteryx velutina*.

Drymonia velutina Oberthür, Etud. Ent. V. p. 64, pl. 8. fig. 2 (1880) ;

STAUDINGER, Cat. Lep. pal. p. 110 (1901) ; WILEMAN, Trans. Ent. Soc.

Lond. 1911, p. 296 ; GRÜNBERG, Seitz, Macrolep. II. p. 307, pl. 46h (1912).

Local distribution. Tobetsu, Hakodate, Nikko.

General distribution. Japan, Siberia.

Time of appearance. June.

Genus *Lophontesia* Staudinger (1892).

Palpi porrect or oblique extending to beyond frons, thickly scaled, 3rd joint moderate and obtuse ; proboscis feebly developed ; antennæ in male

pectinate to the tip, in female dentate and ciliated; eyes naked. Legs hairy;



Text-fig. 12. Foretibia of *L. pryeri*, ♂. × 23.

foretibiæ with the lobe extending to just before the end, hindtibiæ with two pairs of spurs. Forewing with termen slightly crenulate; a tuft of scales on inner margin; vein 5 from above middle

of discocellulars; 6 from close upper angle of cell; 7, 8, 9 and 10 stalked, 8 and 9 very short; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Early stages unknown.

Geographical distribution. Palearctic.

Closely allied to *Odontosia* and *Lophopteryx*, but readily distinguishable from them by the absence of the areole.

37. *Lophontosia cuculus*.

(Pl. XXII, fig. 20.)

Odontosia cuculus Staudinger, Rom. Mém. III. p. 226, pl. 17. fig. 5 (1887);

Cat. Lep. pal. p. 110 (1901); WILEMAN, Trans. Ent. Soc. Lond. 1911 p. 294; GRÜNBERG, Seitz. Macrolep. II. p. 306, pl. 46d, pl. 49b (1912).

Local distribution. Tobetsu, Shinano.

General distribution. Japan, Siberia.

Time of appearance. August.

WILEMAN considers that this species may be identical with the following species *L. pryeri*, and I was also of the same opinion. At present, however, I consider that both are the distinct species. In *cuculus* the forewing of ♂ has a larger and more distinct white spot on the inner margin beyond the middle, and the antennæ of the male has the shorter branches. In *pryeri* the white spot on the inner margin of the forewing of ♂ is smaller and less distinct, and the antennæ of the male has the longer branches.

38. *Lophontosia pryeri*.

(Pl. XXII, fig. 19; Pl. XXVII, fig. 16; Pl. XXXIII, fig. 6;
Text-fig. 12.)

Lophopteryx pryeri Butler, A. M. N. H. (15) IV. p. 355 (1879); LEECH, Trans.
Ent. Soc. Lond. 1898, p. 313; GRÜNBERG, Seitz, Macrolep. II. p. 307
(1912).

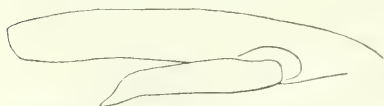
Local distribution. Tokyo, Yokohama, Oiwake, Nachi, Hokkaido.

Habitat. Japan.

Time of appearance. July.

Genus *Allodonta* Staudinger (1887).

Palpi obliquely porrect, first two joints thickly scaled, 3rd moderate and rather obtuse; proboscis present; antennae in male pectinate to two-thirds; eyes naked. Thorax with a tuft of scales. Legs hairy; foretibiae with the lobe extending to four-fifths, hindtibiae with two pairs of spurs. Forewing with a tuft of scales on inner margin; vein 5 from middle of discocellulars; 6



Text-fig. 13. Foretibia of *A. leucodera*, ♂. ×23.

from upper angle of cell or shortly stalked with 7, 8, 9 and 10; no areole. Hindwing with veins 3 and 4 from lower angle of cell; 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Early stages unknown.

Geographical distribution. Palearctic.

Resembles in the general appearance to the genus *Hyperaschra*, but readily distinguished from it by the venation and the palpi.

39. *Allodonta leucodera*.

(Pl. XXIII, fig. 13; Pl. XXV, fig. 7; Pl. XXIX, fig. 1;
Pl. XXXV, fig. 3; Pl. XXXIX, fig. 8; Text-fig. 13.)

Allodonta leucodera Staudinger, Rom. Mém. VI. p. 357 (1892); Cat. Lep. pal.
p. 109 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 303, pl. 46b (1912).

Hypereschna collaris Swinhøe, A.M.N.H. XIV. p. 132 (1904).

Local distribution. Nikko, Yamato, Junsai Numa, Hakodate.

General distribution. Japan, India, Siberia.

Time of appearance. May, June, July, September.

*40. *Allodonta gigantea*.

Peridea gigantea Butler, A. M. N. H. (4) XX. p. 474 (1877); Ill. Het. B.M.

II. p. 12, pl. 24. fig. 6 (1878); KIRBY, Cat. Lep. Het. p. 600 (1892);

LEECH, Trans. Ent. Soc. Lond. 1898, p. 311; WILEMAN, Trans. Ent. Soc.

Lond. 1911, p. 290; GRÜNBERG, Seitz, Macrolep. II. p. 302, pl. 46b (1912).

? *Allodonta plebeja* Oberthür, Etud. Ent. V. p. 65, pl. 8. fig. 7 (1880).

Local distribution. Yoshino, Nikko, Tokyo, Tobetsu, Shiokubi.

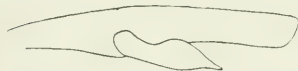
General distribution. Japan, Siberia?

Time of appearance. June, August.

WILEMAN considers that *Allodonta plebeja* is identical with *Peridea gigantea* Butl., but OBERTHÜR's figure of *plebeja* differs greatly from BUTTER's figure of *gigantea*.

Genus **Yazawaia** n. gn.

Palpi porrect and woolly haired, 3rd joint small; proboscis present; antennæ in male dentate and fasciculate, in female ciliated; eyes naked. Legs hairy; foretibiae with the lobe extending to about two-thirds, hindtibiae



Text-fig. 14. Foretibia of *Y. japonica*, ♂. × 23.

with two pairs of spurs. Forewing rather broad, with the costa slightly arched; inner margin with a tuft and strongly excised beyond middle; vein 5 from just above middle of discocellulars; 6 from upper angle of cell or stalked with 7, 8, 9 and 10; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell and connected with it by a short bar.

Early stages unknown.

Type. *Y. japonica*.

Allied to *Ochrostigma*, but distinguished from it by the costa of forewing being arched and the inner margin of the forewing being strongly excised beyond middle.

41. *Yazawaia japonica* n. sp.

(Pl. XXIII, fig. 14; XXV, figs. 4, 8; Pl. XXIX, fig. 2;

Pl. XXXV, fig. 4; Text-fig. 14.)

Palpi, head and thorax blackish brown, tegulae mixed with whitish; abdomen brownish grey. Forewing purplish black with a pale ochreous or red-brown patch at apex, anal angle and base below cell; a double antemedial waved blackish line, filled in with red-brown, strongly curved outwards in cell, the inner line indistinct; a blackish streak on discocellulars defined by red-brown; a double postmedial dentate line outwardly curved between veins 4 and 6, the inner line black and the outer red-brown; tuft of scales on inner margin red-brown; a subterminal series of red-brown specks indistinct; two longitudinal black streaks on the basal patch; terminal line black; cilia blackish brown with a whitish spot at end of vein. Hindwing fuscous grey, with an indistinct dentate diffused dark postmedial line; terminal line blackish; cilia fuscous with a pale line at base. Underside fuscous grey, with traces of postmedial line on both wings; forewing with the one-third of costa towards apex tinged with ochreous.

Expanse. ♂ 41 mm., ♀ 42 mm.

Type. A male specimen taken by Yazawa on Mt. Shirouma, Shinano, August 5th, 1916.

I have seen a female specimen taken by TAKENOUCHI on Mt. Yatsugatake, Shinano, August 20th 1915.

Allied to *Hypereschna tenebrosa* and *Allodonta leucodera*.

Genus **Cerura**¹ Schrank (1802).

Dicranura Boisduval (1829).

Palpi slight, porrect and woolly haired, 3rd joint slightly longer than 2nd; proboscis vestigial; antennae pectinate to the tip in both sexes, the branches

1. As the type species of *Cerura* is *C. milhauseri* the generic name *Dicranura* should be adopted.

long in male, short in female; eyes naked. Body and legs woolly haired; foretibiæ with the lobe very long, reaching far beyond the end; hindtibiæ with a pair of short terminal spurs. Forewing without tuft of scales on inner



Text-fig. 15. Foretibia of *C. vinula* ♂. ×23.

margin; vein 5 from middle of discocellulars or from just below upper angle of cell; 6, 7, 8 and 9 stalked; 10 from cell and anastomosing with the stalk of 7, 8 and 9 to form a small areole. Hindwing with vein 5 from middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ smooth and naked, 3rd segment slightly humped above; anal legs prolonged into long slender processes, in which slender reddish filaments are concealed. Cocoon very hard formed on twigs.

Pupæ with apex pointed, anal end rounded.

Geographical distribution. Palearctic, Oriental and Nearctic.

HAMPSON, STANDINGER, MATSUMURA and GRÜNBERG separates the genus *Dicranura* from this genus, while KIRBY, SPULER as well as PACKARD are contrary to it. I can not find any difference sufficient to separate the genus into two, both in imaginal and larval stages, except the length of the stalk of veins 6 and 7 in hindwing and the wing-markings.

Sect. I. Hindwing with veins 6 and 7 stalked to near ends them.

42. *Cerura lanigera*.

(Pl. XXXIV. fig. 8.)

Cerura lanigera Butler, A.M.N.H. (4) XX. p. 474 (1877); Ill. Het. B.M. III. p. 10, pl. 43. fig. 11 (1879); LEECH, Trans. Ent. Soc. Lond. 1898, p. 307; STAUDINGER, Cat. Lep. pal. p. 105 (1901); Grünberg, Seitz, Macrolep. II. p. 286, pl. 44b (1912).

Cerura furcula (part) Leech, (nec CLERCK) P.Z.S. 1888, p. 644.

Cerura bicuspis japonica Grünberg, Seitz, Macrolep. II. p. 286, pl. 44c (1912).

Local distribution. Tokyo, Kaga, Gifu; Yokohama, Shikoku, Corea.

General distribution. Japan, Corea, Siberia.

Time of appearance. May, June, August.

*43. *Cerura bifida*.

Bombyx bifida Hübner, Bomb. pl. 10. fig. 38 (1800?); STAUDINGER, Cat. Lep. pal. p. 106 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 287, pl. 44c (1912).

Local distribution, Hokkaido, Shikoku?

General distribution. Japan, Asia Minor, America, Persia, Altai, Europe

Matsumura has given Honshu as the home of the species, while no body has taken it in the same locality.

44. *Cerura furcula*.

Bombyx furcula Clerck, Icones. pl. 9. fig. 9 (1759); LINNÆUS, Faun. Suec. p. 298 (1761); HÜBNER, Bomb. fig. 39 (1800?); LEECH, Trans. Ent. Soc. Lond. 1898, p. 307; STAUDINGER, Cat. Lep. pal. p. 106 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 286, pl. 44c (1912).

Cerura sangaica Moore, A.M.N.H. (4) XX. p. 90 (1877).

Local distribution. Corea (Gensan).

General distribution. Corea, China, Siberia, Asia Minor, Europe.

Sect. II (*Hieranura*). Hindwing with veins 6 and 7 stalked rather shortly.

*45. *Cerura liturata*.

Cerura liturata Walker, Cat. V. p. 988 (1855); Butler, Ill. Het. B. M. VI. p. 19, pl. 106. fig. 7 (1880); Hampson, Moths Ind. I. p. 155 (1892).

Local distribution. Formosa.

General distribution. Formosa, China, India.

46. *Cerura erminea*.

(Pl. XXII, fig. 13.)

Bombyx erminea Esper, Schmett. III. p. 100, pl. 19. figs. 1, 2 (1784);

HÜBNER, Bomb. fig. 35; KIRBY, Cat. Lep. Het. p. 588 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 308; STAUDINGER, Cat. Lep. pal. p. 106 (1901); GRÜNBERG,

Seitz, Macrolep. II. p. 287, pl. 44d (1912).

Cerura menciaana Moore, A.M.N.H. (4) XX. p. 89 (1877).

Dicranura erminia var. *menciaana* STAUDINGER, Cat. Lep. pal. p. 106 (1901).

Local distribution. Suigen, (Corea).*

General distribution. Corea, China, Siberia, Europe.

Time of appearance. August.

I have received a pair of this species from . ITO.

47. *Cerura vinula*.

(Pl. XXIX. fig. 8; XXXIV. fig. 7; Text-fig. 15.)

Bombyx vinula Linnaeus, Syst. Nat. I. p. 499 (1758); HÜBNER, Bomb. pl. 9. fig. 34, KIRBY, Cat. Lep. Het. p. 588 (1892); HAMPSON, Moths Ind. I. p. 157 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 308; STAUDINGER, Cat. Lep. pal. p. 106 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 288, pl. 44b (1912).

Dicranura felina Butler, A.M.N.H. (4) XX. p. 474 (1877).

Dicranura vinula var. *felina* Staudinger, Cat. Lep. pal. p. 107 (1901).

Dicranura askolda Oberthür, Etud. Ent. V. p. 59, pl. 8. fig. 8 (1880).

Local distribution. Tokyo, Hokkaido, Formosa, Corea.

General distribution. Japan, Corea, Formosa, Siberia Europe.

Time of appearance. April, May, July.

Egg flat, oval, pal red-brown, reticulated with darker, a black spot at apex. Diametre 1.5 mm.

Larvæ. 1st stage. Head black, body dark grey-brown; first segment bears two black short processes with several small spines; suranal plate black; dorsal line dark, but indistinct; each segment sparsely bears short hair. Length of body 6 mm.; of anal process 3 mm.

2nd stage. Body yellowish green; head, processes on 1st segment and suranal plate black; 1st, 2nd and 3rd segments grey-brown above, with a dorsal line of yellowish green excepting the 3rd segment; from 4th to terminal

segments dark grey-brown above, with a darker dorsal line; subdorsal line only distinct on 1st three segments; suprspiracular and spiracular lines purplish brown and interrupted; a feeble subspiracular line sometimes present; anal processes purplish black with an yellowish brown ring beyond middle; dark grey-brown marking on the dorsum narrow on segments 3rd, 4th and 10th. Length of body 9 mm.; of anal process 6 mm.

3rd stage. Yellowish green; stigmata and dorsal line distinct; subdorsal, spiracular, supra- and sub-spiracular lines disappear; dorsal band blackish brown, narrowest on 3rd, broadest on 7th segment; there is a dark line on the dorsal band, but indistinct excepting the 1st and 3rd segments, on which the line is represented by the ground colour; anal processes purplish black with a brown ring. Length of body 24 mm.; of anal process 7 mm.

4th stage. Yellowish green; dorsal band purplish brown reticulated with black, edged on both sides with black and yellow; a red ring around head distinct. Length of body 32 mm.; of anal process 8 mm.

5th stage. Head brown, laterally blackish brown; dorsal band white reticulated with blue, edged on both sides with dark purplish and white dorsal hump on 3rd segment very small and purple; processes on 1st segment disappear, leaving only a trace tinged with brown; anal processes pale blue with black spines. Shortly before their pupation the dorsal band becomes reddish brown. Length of body 45 mm.; of anal process 10 mm.

Larvæ spin a very hard brownish cocoon on branch or trunk of a tree, on which they feed.

Pupæ with vertex somewhat pointed; anal end bears short spines; body brownish black; head purplish black. Length 24 mm.

Food plants. *Salix babylonica*, *Populus* etc.

Genus **Macrurocampa** Dyar (1893).

? *Disparia* Nagano (1916).

Palpi short and porrect, third joint small or moderate and rather obtuse or rounded; proboscis feebly developed; antennæ in male pectinate to beyond middle or serrate and fasciculate, in female ciliated; eyes naked sometimes overhung by cilia. Head and thorax thickly haired with a tuft of rough hair

on the vertex. Legs hairy; foretibiae with the lobe extending to or just



Text-fig. 16. Foretibia of *M. sigmata* ♂. ×23.

before the end, hindtibiae with two pairs of spurs. Forewing without tuft of scales on inner margin; vein 5 from middle or from above middle of discocellulars; 6, 7, 8, 9 and 10 stalked;

11 free; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

The early stages of the Japanese species of the genus are unknown to me excepting those of *Macrurocampa variegata* which differ from those of *M. marthesia*.

Geographical distribution. Palearctic, Oriental, Nearetic, Neotropical.

Sect. I. Antennæ in male serrate and fasciculate.

48. *Macrurocampa sigmata*.

(Pl. XXII, fig. 6; Pl. XXVI, fig. 3; Pl. XXXI, fig. 7;

Pl. XXXV, fig. 6; Text-fig. 24.)

Phalera sigmata Butler, A.M.N.H. (4) XX. p. 473 (1877); Ill. Het. B.M. II. p.

11, pl. 24. fig. 9 (1878); LEECH, Trans. Ent. Soc. Lond. 1898, p. 299;

GRÜNBERG, Seitz, Macrolep. II. p. 313, pl. 472 (1912).

Cnethodonta (Somera) pryori Matsumura (nec Leech), Zoku-senchu-zukai. I. p.

54, pl. 9. fig. 8 (1909).

Local distribution. Usuki, Tokyo, Hakone, Yokohama, Hakodate.

General distribution. Japan, China.

Time of appearance. June, July.

49. *Macrurocampa delia*.

(Pl. XXXIX, fig. 4.)

Drymonia delia Leech. P. Z. S. 1888, p. 640, pl. 32 fig. 3; Trans. Ent. Soc.

Lond. 1898, p. 303; GRÜNBERG, Seitz, Macrolep. II. p. 297, pl. 45d (1912).

Local distribution. Tokyo, Oiwake, Kaga.

Habitat. Japan.

Time of appearance. August.

Sect. II. Antennæ in male pectinate.

50. *Macrurocampa nipponica*.

Fentonia nipponica Wileman, Trans. Ent. Soc. Lond. 1911, p. 286. pl. 30.

fig. 5; GRÜNBERG, Seitz, Macrolep. II. p. 292 (1912).

Drymonia eximia Grünberg, Seitz, Macrolep. II. p. 297, pl. 45d (1912).

Local distribution. Nikko, Yoshino.

Habitat. Japan.

Time of appearance. August.

51. *Macrurocampa variegata*.

(Pl. XXVI, fig. 6; Pl. XXVII, fig. 4; Pl. XXX. fig. 8;

Pl. XXXVIII, fig. 3.)

Fentonia variegata Wileman, Entom. 1910, p. 290.

Fentonia sordita Wileman, Trans. Ent. Soc. Lond. 1911, p. 286, pl. 30. fig. 8;

GRÜNBERG, Seitz, Macrolep. II. p. 292 (1912); NAGANO, Bull. Nawa: Ent.

Lab. I. p. 3. pl. 1. figs. 14-26; pl. 9. fig. 17 larva (1916).

Fentonia variegata ab. *formosana* Wileman, Entom. 1910, p. 290.

Local distribution. Kyoto, Gifu, Nachi, Yoshino, Usuki, Kanshirei.

General distribution. Japan, Formosa.

Time of appearance. May, June, July, August.

*52. *Macrurocampa nigrofasciata*.

Fentonia nigrofasciata Wileman, Entom. 1910, p. 290.

Local distribution. Arizan.

Habitat. Formosa.

Time of appearance. August.

Genus *Uropya* Staudinger (1892).

Palpi slight, 2nd joint shorter than 1st, 3rd small; antennæ in male pectinate to beyond middle, in female "setiform with very short teeth" (GRÜNBERG);

proboscis vestigial; eyes naked., Head and thorax densely haired; abdomen long. Legs hairy; foretibiæ with the lobe extending to the end, hindtibiæ



Text-fig. 17. Palpus of *U. meticolodina*, ♂, x37.



Text-fig. 18. Foretibia of *U. meticolodina*, ♂, x23.

with two pairs of spurs. Forewing elongate, with the apex acute; termen slightly dentate; vein 5 from middle of discocellulars; 6 from upper angle of cell or shortly stalked with 7, 8, 9 and 10; 10 connected with the stalk of 8 and 9 to form a small areole by a short bar at the point, where vein 7 arises. Hindwing with vein 5 from just below middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ "with oval head, which is deeply incised above in heart-shape, the incision being much deeper than in *Hoplitis*, the body increasing in size to segment 9, the last two segments again thinner, on segment 11 a wart-like tubercle; anal fork similar to that of *Dicranura* (*Cerura*), but much shorter" (GRÜNBERG).

53. *Uropgia meticolodina*.

(Pl. XXII, fig. 5; Pl. XXXII, fig. 2; Text-figs. 17, 18.)

Notodonta meticolodina Oberthür, Etud. Ent. X. p. 16, pl. 1. fig. 3 (1884); KIRBY, Cat. Lep. Het. p. 606 (1892); STAUDINGER, Rom. Mém. VI. p. 344, pl. 4. fig. 8, larva (1892); Cat. Lep. pal. p. 107 (1901); LEECH, Trans. Ent. Soc. Lond. 1898, p. 305; GRÜNBERG, Seitz, Macrolep. II. p. 293, pl. 45a (1912).

Local distribution. Kaga, Hokkaido.

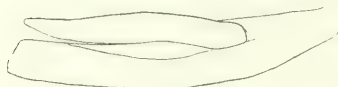
General distribution. Japan, Siberia.

Time of appearance. August.

Genus **Fentonia** Butler (1881).

Palpi upturned to the centre of frons or porrect, 3rd joint moderate and

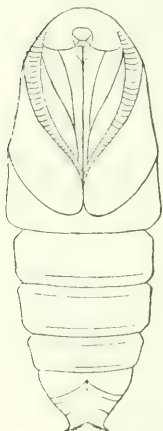
truncate at the tip; proboscis present; antennae in male pectinate to middle, in female setiform; eyes naked.



Text-fig. 19. Foretibia of *F. ocypete*, ♂, ×23.

Abdomen long in male. Legs hairy; foretibiae with the lobe extending to the end, hindtibiae with two pairs of spurs. Forewing elongate, without

tuft of scales on inner margin; vein 5 from above middle of discocellulars; 6, 7 and 8 stalked; 9 and 10 stalked, 9 anastomosing with 8 to form a long areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.



Text-fig. 20. Pupa of *F. ocypete*. a. cremaster.

Larvæ with 16 feet, smooth, slightly humped on 8th segment, and taper posteriorly. Spin a rough cocoon under ground.

Papæ with vertex rounded; cremaster bifurcate and dentate.

54. *Fentonia ocypete*.

(Pl. XXIII, figs. 8, 27, 28, 29; Pl. XXVII, fig. 2; Pl. XXIX, fig. 3; Pl. XXXIII, fig. 7; Text-figs. 19, 20.)

Harpyia ocypete Bremer, Bull. Acad. Petersb. 1861, p. 481; Lep. Ost-Sib. p. 44, pl. 5. fig. 1 (1864); OBERTHÜR, Etud. Ent. V. p. 60, pl. 8. fig. 6 (1880); HAMPTON, Moths Ind. I. p. 148 (1892); KIRBY, Cat. Lep. Het. p. 562 (1892); STAUDINGER, Rom. Mém. VI. p. 343 (1892); Cat. Lep. pal. p. 107 (1901); LEECH, Trans. Ent. Soc. Lond. 1898, p. 304; GRÜNBERG, Seitz. Macrolep. II. p. 291, pl. 45b (1912).

Fentonia lavis Butler, Trans. Ent. Soc. Lond. 1881, p. 20.

Local distribution. Usuki, Yamato, Yokohama, Oiwake.

General distribution. Japan, China, Siberia, India.

Time of appearance. June, July, August.

Larvæ (full grown) naked, smooth more or less humped above on segments 8th and 11th, and tapering posteriorly. Head milky white with several longitudinal black and purplish lines; first three segments greyish yellow with a white dorsal line which is edged with purplish black and yellow; the following segments with many irregular black and reddish lines; segments 6th-8th and 10th-12th darker above, an elliptical yellow spot on 7th segment and two quadrate yellow spots transversely on each segment, 9th and 10th. Length 40 mm.

Pupæ dark reddish purple with the head black; body dotted with small punctures. Length 24 mm.

HIRAYAMA took a larva, August 21st, 1915, which soon pupated and emerged, September 10th, 1915. He also took a nearly full grown larva October 28th, 1915. According to his observation, the species passes yearly two generations.

Food plants. *Quercus*.

Genus **Nerice** Walker (1855).

Palpi obliquely upturned, 3rd small and rather pointed; antennæ pectinate to the tip in both sexes; proboscis feeble. Thorax with a high erect crest of hair. Legs hairy; foretibiae with the lobe extending to five-sixths, hindtibiae with two pairs of spurs. Forewing with vein 5 from middle of discocellulars; 6, 7, 8 and 9 stalked; 10 from cell and anastomosing with the stalk of 8 and 9 to form an areole. Hindwing with vein 5 from middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larva «differs from any other genus of the family, in the abdominal segments 1-8 having each a "large anteriorly directed prominence ending in a bifid ridge, the incision being transverse, the anterior portion being curved backwards and larger than the posterior part, the two looking very much like the bill of an eagle, susceptible of being opened and closed" (MARRATT). Pupa. Body rather stout, somewhat pointed at the end, which bears an unusually long, slender, smooth, rounded cremaster, armed with very short curled

setae, and ends in two upcurved slender, hooks. Cocoon formed of thick, brownish silk, situated within folded leaves or under some slight protection at the surface of the soil. Concealed by particles of soil» (PACKARD).

55. *Nerice davidii*.

(Pl. XXIII, fig. 11; Pl. XXXVIII, fig. 7.)

Nerice davidii Oberthür, Etud. Ent. VI. p. 17, pl. 9. fig. 2 (1881); LEECH, Trans. Ent. Soc. Lond. 1898, p. 318; STAUDINGER, Cat. Lep. pal. p. 108 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 295, pl. 45b (1912).

Nerice davidis Matsumura (nec OBERTHÜR), Zoku-seuchin-zukai. I. p. 72, pl. 11. fig. 5 (1909).

Nerice bidentata Leech (nec Walker), P.Z.S. 1888, p. 638.

Local distribution. Shinano, Sapporo, Hakodate.

General distribution. Japan, China, Siberia.

Time of appearance. August.

56. *Nerice? bipartita*.

(Pl. XXV, fig. 10; Pl. XXX, fig. 12.)

Nerice bipartita Butler, Cist. Ent. III. p. 119 (1885); LEECH, Trans. Ent. Soc. Lond. 1898, p. 318; STAUDINGER, Cat. Lep. pal. p. 108 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 295, pl. 45b (1912).

Nerice upina Alphéraky, Rom. Mém. VI. p. 17, pl. 1. fig. 7 (1892).

Local distribution. Nikko, Kyoto, Shikoku, Sapporo.

General distribution. Japan, China.

Time of appearance. June.

Genus *Brachionycoides* n. gn.

Palpi porrect or oblique, 3rd joint moderate and pointed; proboscis present; antennae in both sexes fasciculate; eyes naked. Legs hairy; foretibiae with the lobe extending to about three-fourths, hindtibiae with two pairs of spurs.



Text-fig. 21. Foretibia of *B. atrocittatum*, ♂. × 23.

Forewing without tuft of scales on inner margin; vein 5 from middle of dis-

cocellulars; 6, 7, 8 and 9 stalked; 10 from cell and anastomosing with 7, 8 and 9 to form an areole. Hindwing with vein 5 from middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Early stages unknown.

Type. *B. atrovittatum*.

Geographical distribution. Palearctic.

57. *Brachionycoides atrovittatum*.

(Pl. XXII, fig. 9; Pl. XXV, fig. 3; Pl. XXVII, fig. 13;

Pl. XXIX, fig. 6; Pl. XXXIII, fig. 1; Text-fig. 21.)

Brachionycha atrovittatus Bremer, Bull. Acad. Petersb. II. p. 483 (1861); Lep. Ost-Sib. p. 46, pl. 5, fig. 4 (1864); KIRBY, Cat. Lep. Het. p. 562 (1892); LEECH, Trans. Ent. Soc. Lond. 1893, p. 304; STAUDINGER, Cat. Lep. pal. p. 109 (1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 284; Grünberg, Seitz, Macrolep. II. p. 299, pl. 45f (1912).

Destolmia insignis Butler, Trans. Ent. Soc. Lond. 1881, p. 19.

Notodonta toddii Holland, Trans. Amer. Ent. Soc. XVI. p. 73 (1889).

Local distribution. Tokyo, Nachi, Yoshino, Yokohama, Nikko, Tobetsu, Junsai Numa.

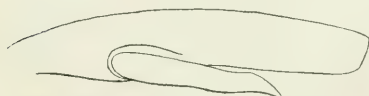
General distribution. Japan, Siberia,

Time of appearance. June—August.

Genus *Hyperæschra* Butler (1880).

Semidonta Standinger (1892).

Palpi upturned, 1st joint hairy, 3rd moderate and obtuse; proboscis feeble; antennæ in male pectinate to before the tip, in female pectinate or ciliated; eyes naked. Legs hairy; foretibiæ with the lobe extending to three-fourths or four-fifths, hindtibiæ with two pairs of spurs. Forewing with a tuft of scales on inner margin; vein 5 from middle of discocellulars;



Text-fig. 22. Foretibia of *H. tenebrosa*?, ♂. ×23.

6, 7, 8 and 9 stalked or 6 from upper angle of cell; 10 from cell and anastomosing with 8 and 9 to form an

areole. Hindwing with vein 5 from about middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Early stages unknown.

Geographical distribution. Palearctic, Oriental and Nearctic.

58. *Hyperaeschra biloba*.

(Pl. XXII, fig. 23; Pl. XXIX, fig. 5; Pl. XXXVI, fig. 6.)

Drymonia biloba Oberthür, Etud. Ent. V. p. 63, pl. 8. fig. 1 (1880); STAUDINGER, Rom. Mém. VI. p. 358 (1892); Cat. Lep. pal. p. 109 (1901); WILEMAN Trans. Ent. Soc. Lond. 1911, p. 295; GRÜNBERG, Seitz, Macrolep. II. p. 302, pl. 45d (1912).

Local distribution. Nikko, Kii, Yoshino, Kaga, Tobetsu, Junsai Numu.

General distribution. Japan, Siberia.

Time of appearance. July, August.

Foretibiae with the lobe extending to three-fourth length of them.

The angulation of the postmedial line of the forewing is modified among the sexes. In the male the postmedial line more deeply incurved between veins 1 and 4 than in the female, and the angulation at vein 1 acute, the space between the ante- and postmedial lines being broader.

The specimens of both sexes taken by HIRAYAMA at Zyozankei, Hokkaido, on August 13th, 1918, differ from OBERTHÜR'S figure in the darker colour and the angulation of the postmedial line. But the specimen are so imperfect that I can not describe them as distinct.

59. *Hyperaeschra basilinea*.

(Pl. XXVI, fig. 5; Pl. XXXIX, fig. 3.)

Notodonta basilinea Wileman, Trans. Ent. Soc. Lond. 1911, p. 292, pl. 30. fig. 23; GRÜNBERG, Seitz, Macrolep. II. p. 302 (1912).

Local distribution. Yoshino, Kyoto.

Habitat. Japan.

Time of appearance. June, August.

60. *Hyperæschra taiwana* n. sp.

(Pl. XXII, fig. 1.)

Palpi brownish grey, fuscous above; head, thorax and abdomen brownish grey. Forewing grey-brown with the basal two-thirds darker; a black longitudinal streak in cell and a double black longitudinal streak below cell on submedian fold, filled in with whitish; a black discocellular streak; a deeply dentate postmedial line defined by whitish on outer side, oblique from costa to vein 4, then inwardly oblique and indistinct; an indistinct subterminal dentate line, defined by whitish on outer side; veins finely streaked with black; cilia grey-brown with a pale line at base. Hindwing whitish suffused with brown towards termen; cilia whitish mixed with brown. Underside of forewing grey-brown with an almost straight dark postmedial line; of hindwing whitish tinged slightly with brownish.

Expanse. ♂ 50 mm.

Type. A male specimen taken at Horisha, Formosa, August 7, 1917.

Local distribution. Horisha.

Habitat. Formosa.

?61. *Hyperæschra tenebrosa*.

(Pl. XXII, fig. 24; Pl. XXVI, figs. 2, 8; Pl. XXIX, fig. 4;

Pl. XXXVIII, fig. 2; Text-fig. 22)

Phalera tenebrosa Moore, P.Z.S. 1865, p. 815; HAMPSON, Moths Ind. I. p. 164 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 312.

Local distribution. Yokohama, Kaga.

General distribution. Japan, India.

Time of appearance. August.

Antennæ of male serrate, palpi with 1st joint rather long, and foretibiae with the lobe extending to four-fifths length of them. I have doubtfully identified the specimen figured with this species.

Genus *Notodonta* Ochsenheimer (1810).

Palpi porrect, not reaching beyond frons, 1st joint long and almost equal in length to 2nd, 3rd rather long and pointed; proboscis vestigial; antennæ

in male pectinate to about one-fourth, in female serrate; eyes hairy. Legs hairy, especially on forelegs; foretibiae with the lobe extending to about three-



Text-fig. 23. Foretibia of *N. dembowskii*, ♂. x23

fourths, hindtibiae with two pairs of spurs. Forewing with a tuft of scales on inner margin; vein 5 from middle of discocellulars; 6, 7, 8, 9 and 10 stalked; 11

free; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ with "head large, square; a large high nutant hump on second and a lower one on third and a very prominent one on eighth abdominal segment, the latter ending in two tubercles. Anal legs long, but used in walking. The European species have from three to five humps. In the European *N. ziczac* there are, judging by Buckler's figures, as in our species, but three humps; in *N. tritophus* there are four, while the larva of *N. dromedarius* most approaches *Nevice* in having five humps, four on each of the four basal abdominal segments and one on the eighth" (Packard).

Pupæ with "no distinct cremaster, the body being smooth and rounded at the end" (Packard).

Geographical distribution. Palearctic, Oriental and Nearectic.

62. *Notodonta dembowskii*.

(Pl. XXVII, fig. 1; Pl. XXX, fig. 9; Pl. XXXIX, fig. 2;

Text-fig. 23.)

Notodonta dembowskii Oberthür, Diagn. p. 11 (1879); Etud. Ent. V. p. 62, pl. 2, fig. 4 (1880); STAUDINGER, Cat. Lep. pal. p. 109 (1901); WILEMAN. Trans. Ent. Soc. Lond. 1911, p. 291; GRÜNBERG, Seitz, Macrolep. II, p. 300, pl. 45g (1912).

Local distribution. Nikko, Usuitoge, Tobetsu, Junsai Numa.

General distribution. Japan, Siberia.

Time of appearance. May, June, July, August.

63. *Notodonta stigmatica*.

(Pl. XXII. fig. 15.)

Hypodonta pulcherrima ab. *stigmatica* Grünberg, Seitz, Macrolep. II. p. 299, pl. 45g (1912).

Notodonta dembowskii (part) Wileman, Trans. Ent. Soc. Lond. 1911, p. 291.

?*Notodonta rothschildi* Wileman, Entom. 1916, p. 133.

Local distribution. Tobetsu.

Habitat. Japan (Hokkaido).

Time of appearance. July, August.

This species should belong to the genus *Notodonta* instead of *Hypodonta*. The absence of the tuft of scales on the inner margin of forewing as figured by Grünberg is probably due to the examination of an imperfect specimen. Moreover he did not mention the sex, the weak pectination of antennæ shown in his figure makes me conclude that it is certainly a male of *Notodonta* but not *Hypodonta* as he refers to, since in the latter the antennæ of the male are strongly pectinate while in the female they are ciliated.

Notodonta rothschildi WILEMAN may possibly be identical with the present species.

64. *Notodonta tritophus*.

Bombyx tritophus Esper, Schnett. III. p. 279, pl. 60. figs. 1, 2 (1786);

HÜBNER, Bomb. fig. 29; STAUDINGER, Cat. Lep. pal. p. 109 (1901);

WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 289; GRÜNBERG, Seitz, Macrolep. II. p. 301, pl. 46a (1912).

Bombyx torva Hübner, Bomb. p. 108 (1800?)

Local distribution. Sapporo, Hakodate.

General distribution. Japan, Siberia, Europe.

*65. *Notodonta cinerea*.

Peridea cinerea Butler, A. M. N. H. (5) IV. p. 353 (1878); KIRBY, Cat. Lep.

Het. p. 600 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 310;

GRÜNBERG, Seitz, Macrolep. II. p. 302 (1912).

Local distribution. Yokohama, Gifu, Hakodate.

Habitat. Japan.

Time of appearance. August.

*66. *Notodonta griseotincta*.

Notodonta griseotincta Wileman, Entom. 1910, p. 312.

Local distribution. Rantaizan.

Habitat. Formosa.

Time of appearance. May.

*67. *Notodonta furva*.

Notodonta furva Wileman, Entom. 1910, p. 313.

Local distribution. Kanshirei.

Habitat. Formosa.

Time of appearance. April.

*68. *Notodonta? basinotata*.

Notodonta? basinotata Wileman, Entom. 1910, p. 344.

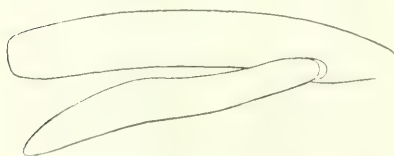
Local distribution. Kanshirei.

Habitat. Formosa.

Time of appearance. April.

Genus **Hupodonta** Butler (1877).

Palpi obliquely upturned, 3rd joint long and somewhat pointed; proboscis fully developed; antennae in male pectinate to near the tip, in female ciliated;



Text-fig. 24. Foretibia of *H. pulcherrima corticalis*. ♂. ×23.

eyes naked. Legs hairy; foretibiae with the lobe extending to the end, hindtibiae with two pairs of spurs. Forewing elongate, without tuft of scales on inner margin; vein 5 from middle of discocellulars; 6, 7,

8, 9 and 10 stalked; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ with a long pointed process on the segments 2nd, 7th and 11th, the process on 7th segment longest.

Pupæ with the cremaster prolonged and pointed.

Geographical distribution. Palearctic and Oriental.

69. *Hupodonta pulcherrima*.

(Pl. XXIII, fig. 33; Pl. XXX, fig. 6; Pl. XXXVIII, fig. 4;

Text-fig. 24.)

Anodonta pulcherrima Moore, P.Z.S. 1865, p. 814, pl. 43, fig. 4; HAMPSON, Moths Ind. I. p. 161 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 309; STAUDINGER, Cat. Lep. pal. p. 108 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 299, pl. 45g (1912).

Hupodonta corticalis Butler, A.M.N.H. (4) XX. p. 475 (1877).

Hupodonta pulcherrima var. *corticalis* Standinger, Cat. Lep. pal. p. 108 (1901).

Local distribution. Nikko, Tokyo, Yokohama, Kyoto, Hokkaido.

General distribution. Japan, India.

Time of appearance. July, August.

Larvæ. Head bluish green, laterally streaked with white and black; body yellowish green, each segment traversed by blue lines; stigmata reddish brown; subspiracular line white; subdorsal line yellow; oblique lines at the side composed of the series of yellow spots; three dorsal processes crimson streaked with white or yellow. Length about 40 mm. The larvæ have been reared by Yano with the leaves of cherry at Tokyo. The above description of the larvæ is based upon the figure prepared by YANO.

70. *Hupodonta obsoleta* n. sp.

(Pl. XXII, fig. 11.)

More grizzle than the preceding by the reduction of rufous tinge. Forewing whitish grey, a black spot on costa near base; an oblique antemedial triangular black patch on costa, followed by a large patch of the ground colour; inner marginal area below median nervure whitish ochreous; a double slightly dentate blackish postmedial line, bent outwards below costa and indistinct below vein 2; a diffused reddish brown subterminal waved line,

edged outwardly with whitish, suffused inwardly with fawn colour to the postmedial line; terminal area dark grey, with a black line on it; terminal line black; cilia grey-brown. Hindwing whitish, suffused with brown on the outer half; an indistinct postmedial and a blackish terminal lines. Underside whitish suffused and irrorated with brown, traces of a postmedial line on hindwing.

Expanse. ♂ 47 mm., ♀ 59 mm.

Type. A pair of specimens taken by HIRAYAMA at Zyozankei, Hokkaido, August 1918.

Local distribution. Zyozankei (Hokkaido).

Habitat. Japan.

Genus *Pheosia* Hübner (1822?).

Palpi porrect, slight and woolly haired, 3rd joint large; proboscis vestigial; eyes naked; antennae in male pectinate to the tip. Legs hairy, foretibiae and



Text-fig. 25. Foretibia of *P. dictuoides*, ♂, x23.



Text-fig. 26. Foreleg of *P. dictuoides*, ♂, x6.

tarsi fringed with long hair; the lobe of foretibiae not reaching the end, hindtibiae with two pairs of spurs. Forewing elongate with a tuft of scales on inner margin; vein 5 from middle of discocellulars; 6 from upper angle of cell; 7, 8, 9 and 10 stalked; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked to near end; 8 running close along upper margin of cell; a slight veinlet in cell.

Larvae "elongate-cylindrical, smooth and as glossy as porcelain, bearing only dispersed minute hairs; 16 feet; on abdominal segment 8 a broad pyramidal tubercle which ends in a short point; head broad and flat" (Grünberg).

Pupae "in the ground in a silk-lined cell, slender, cylindrical, with a short bifid fork at the anal end" (Grünberg).

Geographical distribution. Palearctic, Oriental and Nearectic.

71. *Pheosia dictaoides*.

(Pl. XXIII, fig. 1; Pl. XXIX, fig. 9; Pl. XXXVIII, fig. 1;

Text figs. 25, 26.)

Bombyx dictaoides Esper, Schmett. III. Cont. p. 27, pl. 84. fig. 3 (1789);
HÜBNER, Bomb. figs. 23, 24; STAUDINGER, Cat. Lep. pal. p. 103 (1901);
GRÜNBERG, Seitz, Macrolep. II. p. 298, pl. 45f (1912).

Local distribution. Shinano (Mt. Yatsugatake).

General distribution. Japan, Siberia, Europe.

Time of appearance. July.

Hitherto unrecorded from Japan.

Genus *Leucodonta* Staudinger (1892).

Palpi porrect and hairy, hardly reaching beyond the frons; proboscis vestigial; antennæ in male pectinate to the tip, the branches short and stiff, in female setiform; eyes naked. Legs hairy; foretibiæ with the lobe extending

Text-fig. 27. Foretibia of *L. bicoloria*, ♂. × 23.

to three-fourths, hindtibiæ with two pairs of spurs. Forewing with a tuft of scales on inner margin; vein 5 from above middle of discocellulars; 6, 7, 8 and 9 stalked; 10 from cell

and anastomosing with 7, 8 and 9 to form an areole. Hindwing with vein 5 from far above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ "smooth, very sparsely hairy, with 16 feet and without tubercles" (Grünberg).

Pupæ "slender, cylindrical, with the anal end rounded" (Grünberg).

Geographical distribution. Palearctic.

72. *Leucodonta bicoloria*.

(Pl. XXIII, fig. 9; Pl. XXXIII, fig. 5; Text-fig. 27.)

Bombyx bicoloria Schiffermüller, Esper, Schmett. III. pl. 41. fig. 7; STAUDINGER, Rom. Mém. VI. p. 349 (1892); Cat. Lep. pal. p. 110 (1901); LEECH,

Trans. Ent. Soc. Lond. 1898, p. 316; GRÜNBERG, Seitz, *Macrolep.* II. p. 304, pl. 46f (1912).

Bombyx bicolora Hübner, *Bomb.* pl. 5. fig. 18.

Local distribution. Kanikochi, Nikko, Fuji-san.

General distribution. Japan, Siberia, Europe.

Time of appearance. July.

Genus *Wilemanus* Nagano (1916).

Palpi short and porrect, 1st two joints thickly scaled, 3rd moderate and obtuse; proboscis present; antennae in male pectinate to two-thirds, in female fasciculate; eyes naked. Legs hairy; foretibiae with the lobe extending to



Text-fig. 28. Foretibia of *W. bidentatus*, ♂. ×23.

the end, hindtibiae with two pairs of spurs. Forewing without tuft of scales on inner margin; vein 5 from middle of discocellulars; 6, 7 and 8 stalked; 9 and 10 stalked,

9 anastomosing with 8 to form an areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ smooth, somewhat resemble to those of *Heterocampa manteo* of N. America, the fourth and eleventh segments slightly humped above.

Pupæ "elongate ellipsoidal with round head; cremaster with two processes" (Nagano).

Geographical distribution. Palearctic.

73. *Wilemanus bidentatus*.

(Pl. XXIII, fig. 24; Pl. XXVII, fig. 3; Pl. XXX, fig. 11;

Pl. XXXIII, fig. 8; Text-fig. 28.)

Stenotopus bidentatus Wileman, Trans. Ent. Soc. Lond. 1911, p. 287, pl. 30.

fig. 9; GRÜNBERG, Seitz, *Macrolep.* II. p. 290 (1912); NAGANO, Bull.

Nawa Ent. Lab. 1. p. 2, pl. 1. figs. 1-13, pl. 9. fig. 19, larva (1916).

Ochrostigma ussuriensis Püngeler, Seitz, *Macrolep.* II, p. 305, pl. 49b (1912).

Local distribution. Usuki, Yoshino, Shizuoka, Corea.

General distribution. Japan, Corea, Siberia.

Time of appearance. May–August.

Genus **Lophodonta** Packard (1864).

Palpi short and porrect or oblique, 3rd joint moderate and obtuse or somewhat pointed; proboscis feebly developed; antennæ in male fasciculate,



Text-fig. 29. Foretibia of *L. græseri*, ♂. ×23.

in female ciliated; eyes naked. Legs hairy; foretibiæ with the lobe extending to about three-fourths, hindtibiæ with two pairs of

spurs. Forewing elongate, with a tuft of scales on inner margin; vein 5 from middle of discocellulars; 6, 7, 8, 9 and 10 stalked; 11 free; no areole, Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ. "Body much as in *Notodonta*, but the head is smaller and it has no such suranal plate, this being small and rounded at the end, while the body is smooth, the skin not granulated. From *Notodonta* it differs in the body being noctuiform, not humped. A faint double median dorsal line and a lateral line; the whole body pea-green. Spins no cocoon" (PACKARD)

Pupæ. "Body full and plump, the end of the abdomen very much rounded and obtuse, with no distinct cremaster" (PACKARD).

Geographical distribution. Palearctic, Oriental? and Nearctic.

74. *Lophodonta græseri*.

(Pl. XXII, fig. 17; Pl. XXVI, fig. 4; Pl. XXX, fig. 7; Text-fig. 29.)

Notodonta græseri Staudinger, Rom. Mém. VI. p. 351, pl. 5. fig. 3 (1892); Cat.

Lep. pal. p. 109 (1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 291; GRÜNBERG, Seitz, Macrolep. II. p. 300, pl. 46 a (1912).

Notodonta Ishida Matsumura, Zoku-senchiu-zukai. I. p. 56, pl. 10. fig. 1 (1909).

Local distribution. Kaga, Yoshino, Sapporo.

General distribution. Japan, Siberia.

Time of appearance. August, September.

75. *Lophodonta aliena*.

Notodonta aliena Staudinger, Rom. Mém. VI. p. 352, pl. 5. fig. 4 (1892); Cat.

Lep. pal. p. 109 (1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 291; GRÜNBERG, Seitz, Macrolep. II. p. 300 pl. 46 a (1912).

Notodonta Nitobei Matsumura, Zoku-senchiu-zukai. I. p. 59, pl. 10 fig. 5 (1909).

Local distribution. Yoshino, Shinano, Aomori.

General distribution. Japan, Siberia.

Time of appearance. June, September.

76. *Lophodonta lativitta*.

Notodonta lativitta Wileman, Trans. Ent. Soc. Lond. 1911, p. 292, pl. 30. fig. 4; GRÜNBERG, Seitz, Macrolep. II. p. 302 (1912).

Local distribution. Yoshino, Shinano.

Habitat. Japan.

Time of appearance. August, September.

77. *Lophodonta monetaria*.

(Pl. XXVI, fig. 7; Pl. XXXVI, fig. 2.)

Notodonta monetaria Oberthür, Etud. Ent. V. p. 62, pl. 2. fig. 6 (1880);

KIRBY, Cat. Lep. Het. p. 560 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 310; STAUDINGER, Cat. Lep. pal. p. 109 (1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 290; GRÜNBERG, Seitz, Macrolep. II. p. 301, pl. 46 b (1912).

Notodonta oberthüri Staudinger, Rom. Mém. VI. p. 354, pl. 5 fig. 5 (1892).

Local distribution. Tokyo, Kaga, Yoshino, Nagahama, Tobetsu.

General distribution. Japan, Siberia.

Time of appearance. May-September.

One of the female examined by me is much darker; hindwing almost fuscous, and the transverse lines and bands are indistinct.

Genus **Euhampsonia** Dyar (1897).

Palpi porrect and thickly scaled, 3rd joint very small; proboscis present; antennæ in male pectinate to two-thirds, in female ciliated; eyes naked, over-



Text-fig. 30. Foretibia of *E. cristata*, ♀. × 12.

overhung by cilia. Thorax with a high erect crest of hair. Legs hairy; foretibiæ with the lobe not extending to the end, hindtibiæ with two pairs of spurs. Forewing with termen crenulate

and irregular above vein 4; tuft of scale on inner margin either large or small; vein 5 from middle of discocellulars; 6 from just above upper angle of cell or just below it; 7, 8 and 9 stalked; 10 anastomosing with the stalk of 8 and 9 to form an areole. Hindwing with vein 5 from middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ smooth, cylindrical, without humps and tubercles; 10 feet. Closely resemble to those of *Nadata*.

Geographical distribution. Palearctic and Oriental.

78. *Euhampsonia cristata*.

(Pl. XXII, fig. 3; Pl. XXXI, fig. 6; Pl. XXXV, fig. 1; Text-fig. 30.)

Trabala cristata Butler, A. M. N. H. (4) XX. p. 480 (1877); Ill. Het. B. M.

II. p. 18, pl. 27. fig. 1 (1878); KIRBY, Cat. Lep. Het. p. 614 (1892);

STAUDINGER, Rom. Mém. VI. p. 367 (1892); LEECH, Trans. Ent. Soc. Lond.

1898, p. 297; GRÜNBERG, Seitz, Macrolep. II. p. 310, pl. 47 b (1912).

Local distribution. Tokyo, Oiwake, Yokohama, Nagahama, Hokkaido.

General distribution. Japan, China, Siberia.

Time of appearance. June, July.

Larvæ. According to Prof. Sasaki's figure, head paler green streaked at sides with white; body green with the dorsal half pale grey; stigmata red ringed with white; segments 4-11 with an oblique white streak at sides; suranal plate yellow streaked with red.

The tuft of scales on inner margin of forewing well developed; vein 6 of forewing from just above upper angle of cell.

79. *Euhampsonia splendida*.

Nadata splendida Oberthür, Etud. Ent. V. p. 65, pl. 5. fig. 6 (1880);
 STAUDINGER, Rom. Mém. VI. p. 366 (1892); Cat. Lep. pal. p. 111
 (1901); LEECH, Trans. Ent. Soc. Lond. 1898, p. 298; WILEMAN, l. c.
 1911, p. 284; GRÜNBERG, Seitz, Macrolep. II. p. 311, pl. 47 c (1912).

Local distribution. Kaga, Shinano, Tobetsu.

General distribution. Japan, China, Siberia.

Time of appearance. July, August.

The tuft of scales on inner margin of forewing less developed, the termen much less irregular than in the preceding; vein 6 of forewing from just below upper angle of cell.

Genus *Gangaridopsis* Grünberg (1912).

Palpi upturned and not reaching vertex of head, 3rd joint indistinct; proboscis feebly developed; antennae in male pectinate to the tip; eyes hairy.



Text-fig. 31. *G. citrina*, ♂, ×23.

Thorax with a high erect crest. Legs hairy; foretibiae with the lobe not reaching the end, hindtibiae with two pairs

of spurs. Forewing with the apex acute; termen crenulate; without tuft of scales on inner margin; vein 5 from middle of discocellulars; 6, 7, 8 and 9 stalked; 10 from cell and anastomosing with 7, 8 and 9 to form an areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell and connected with it by a bar near base.

Early stages unknown.

Geographical distribution. Palearctic.

80. *Gangaridopsis citrina*.

(Pl. XXIII, fig. 10; Pl. XXXVI, fig. 3; Text-fig. 31.)

Gangarides citrina Wileman, Trans. Ent. Soc. Lond. 1911, p. 283, pl. 31.
 fig. 3; GRÜNBERG, Seitz, Macrolep. II. p. 294, pl. 48 h (1912).

Local distribution. Nikko, Nasuno, Ise.

Habitat. Japan.

Time of appearance. August.

Genus *Himeropteryx* Staudinger (1887).

Palpi porrect and hairy, slightly extending beyond the frons, 3rd joint small with rounded apex; proboscis vestigial; antennæ in male pectinate; eyes naked. Thorax and legs hairy. Foretibiæ with the lobe extending to just



Text-fig. 32. Palpus of *H. miraculosa*, ♂. × 37.



Text-fig. 33. *H. miraculosa*, ♂. × 23.

before the end, hindtibiæ with two pairs of spurs. Forewing rather broad with termen slightly crenulate; a tuft of scales on inner margin; vein 5 from above middle of discocellulars; 6 shortly stalked with 7, 8 and 9; 10 from cell and anastomosing with 7, 8 and 9 to form an areole. Hindwing with termen almost rounded; vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Early stage unknown.

Geographical distribution. Palearctic.

81. *Himeropteryx miraculosa*.

(Pl. XXIII, fig. 26; Pl. XXXIX, fig. 6; Text-figs. 32, 33.)

Himeropteryx miraculosa Staudinger, Rom. Mém. III. p. 228, pl. 12. fig. 10 (1887); Cat. Lep. pal. p. 111 (1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 296; GRÜNBERG, Seitz, Macrolep. II. p. 310, pl. 47 c (1912).

Local distribution. Yanagawa (Kiushiu), Junsai Numa.

General distribution. Japan, Siberia.

Time of appearance. September, October.

Takamuku states that the larvæ feed on the leaves of *Acer palmatum*.

Genus **Pygopteryx** Staudinger (1887).

I have not been able to examine the species of this genus.

Geographical distribution. Palearctic.

*82. *Pygopteryx suava*.

Pygopteryx suava Staudinger, Rom. Mém. III. p. 230, pl. 17. fig. 4 (1887);

Cat. Lep. pal. p. 112 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 315, pl. 47 g (1912).

Local distribution. Shinano.

General distribution. Japan, Siberia.

Genus **Melalopha** Hübner (1810).*Ichthyura* Hübner (1827).

Palpi porrect, slender, thickly scaled, 3rd joint small or moderate; antennæ in both sexes pectinate to the tip; eyes hairy. Legs hairy; forelegs with tibiae and tarsi thickly haired; foretibiae with the lobe extending to the end, hindtibiae with two pairs of spurs. Abdomen of male with anal tuft. Forewing without tuft of



Text-fig. 34. Foretibia of *M. anastomosis*, ♂ × 23.

scales on inner margin; vein 5 from middle of discocellulars or from just below upper angle of cell; 6 from upper angle of cell or stalked with 7, 8, 9 and 10; no areole. Hindwing with vein 5 absent; 6 and 7 stalked; 8 running close along upper margin cell.

Larvæ cylindrical, hairy, with 16 feet; segments 4th and 11th with dorsal tubercles. Spin a cocoon between leaves.

Pupæ with the furcate cremaster.

Geographical distribution. Palearctic, Oriental and Nearctic.

83. *Melalopha anastomosis*.

(Pl. XXXVIII, fig. 5; Text-fig. 34.)

Bombyx anastomosis Linnaeus, Syst. Nat. I. p. 506 (1758); HAMPSON, Moths

Ind. I. p. 172 (1892); KIRBY, Cat. Lep. Het. p. 609 (1892); LEECH,

Trans. Ent. Soc. Lond. 1898, p. 317; STAUDINGER, Cat. Lep. pal. p. 112 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 314, pl. 47 f (1912); NAGANO, Bull. Nawa Ent. Lab. I. p. 8, pl. 3. figs. 1-10, pl. 9. fig. 16, larva (1916).

Pygocera anastomosis var. *orientalis* Fixen, Rom. Mém. III. p. 350 (1887).

Local distribution. Tokyo, Gifu, Hokkaido, Corea.

General distribution. Japan, Corea, China, Siberia, India, Europe.

Time of appearance. May, June, August, September.

Palpi with 3rd joint small.

84. *Melalopha anachoreta*.

(Pl. XXIX, fig. 12; Pl. XXXVIII, fig. 6.)

Bombyx anachoreta Fabricious, Mant. Ins. II. p. 120 (1787); HÜBNER, Bomb. pl. 22. fig. 88; HAMPSON, Moths Ind. I. p. 172 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 317; STAUDINGER, Cat. Lep. pal. p. 112 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 314, pl. 47 g (1912); NAGANO, Bull. Nawa Ent. Lab. I. 9, pl. 3. figs. 11-20, pl. 9. fig. 23, larva (1916).

Ichthyura fulgurita Walker, Cat. XXXII. p. 433 (1865).

Local distribution. Tokyo, Gifu, Kii, Yokohama, Shikoku, Hakodate, Corea.

General distribution. Japan, Corea, China, Siberia, India, Europe.

Time of appearance. May-August.

Palpi with 3rd joint moderate.

85. *Melalopha curtuloides*.

Clostera curtuloides Erschoff, Trudy. IV. p. 193 (1870); Rom. Mém. II. pl. 16. fig. 3; STAUDINGER, l. c. VI. p. 373; Cat. Lep. pal. p. 112 (1901); KIRBY, Cat. Lep. Het. p. 609 (1892); GRÜNBERG, Seitz, Macrolep. II. p. 314, pl. 47 f (1912); NAGANO, Bull. Nawa Ent. Lab. I. p. 9, pl. 3. fig. 21 (1916).

Local distribution. Gifu, Shinano.

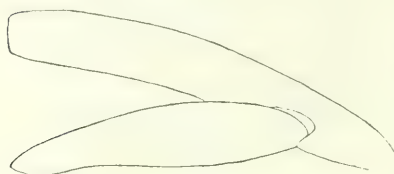
General distribution. Japan, Siberia.

Time of appearance. May, August.

Genus **Egonocia** n. gn.

Palpi obliquely porrect, not reaching beyond frons, thickly scaled, 3rd joint small and obtuse; proboscis vestigial; vertex of head with a tuft of

hairs well developed; antennæ pectinate to before the tip in both sexes, the



Text-fig. 35. Foretibia of *E. cyanea*, ♂. ×23.

branches long in male, short in female; eyes naked. Legs hairy; foretibiae with the lobe extending to or just before the end, hindtibiae with two pairs of spurs. Forewing thickly sealed, subcostal vein hairy on underside; no tuft of scales on

inner margin; vein 5 from middle of discocellulars; 6 from upper angle of cell or stalked with 7, 8, 9 and 10; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell. Both wings with veinlets in the cell.

Type. *E. cyanea*.

Larvæ noctuiform, cylindrical and smooth; 16 feet. Pupate under the ground and spin a rough cocoon.

Pupæ without distinct cremaster.

Geographical distribution. Palearctic and Oriental.

86. *Egonocia cyanea*.

(Pl. XXIII, figs. 5, 30, 31, 32; Pl. XXXI, fig. 4; Pl. XXXVII, fig. 4; Text-fig. 35.)

Somera cyanea Leech, P. Z. S. 1888, p. 642, pl. 32. fig. 5; Trans. Ent. Soc. Lond. 1898, p. 307; GRÜNBERG, Seitz, Macrolep. II. p. 291, pl. 45a (1912).

Local distribution. Tokyo, Kaga, Yokohama.

Habitat. Japan.

Time of appearance. April, May, August.

Larvæ (after Yamada's figure) yellowish green with yellowish dorsal and subdorsal lines; each segment spotted with yellowish; suranal plate red.

Food-plants. *Styrax japonica*.

87. *Egonocia formosana* n. sp.

(Pl. XXIII, fig. 7.)

Whiter than the preceding. Forewing with the greenish tinge much reduced and restricted to the antemedial and postmedial lines, which are more distinct than in the preceding.

Expanse. ♂ 44 mm.

Type. A single male specimen taken at Horisha, Formosa, June 26, 1915.

88. *Egonocia nachiensis* n. sp.

(Pl. XXIII, fig. 6; Pl. XXXVII, fig. 3.)

Palpi dark reddish brown, fringed with whitish; head, thorax and abdomen pale brown irrorated with dark reddish brown, especially towards the end of abdomen. Forewing dark reddish brown, thickly irrorated with bright green at base; the rest of wing sparsely irrorated with white; an indistinct postmedial series of dark spots, mixed with greenish yellow, excurved between veins 3 and 4; cilia dark reddish brown. Hindwing whitish suffused with brown; cilia brownish. Underside whitish; forewing faintly tinged with brown.

Expanse. ♂ 50 mm.

Type. A single male specimen taken at Nachi, Kii, July 28, 1916.

Closely allied to *Stauropus viridipictus* Wileman from Formosa, but distinguishable from it by the forewing being sparsely irrorated with white instead of bright green, and the hindwing paler.

89. *Egonocia fasciata*.

Pheosia fasciata Moore, P. Z. S. 1888, p. 401; BUTLER, Ill. Het. B. M. VII. p. 47, pl. 125. figs. 9, 10; HAMPTON, Moths Ind. I. p. 160 (1892); GRÜNBERG, Seitz, Mocerlep. II. p. 298, pl. 49 a (1912).

Local distribution. Shinano.

General distribution. Japan, India.

Time of appearance. August.

Hitherto unrecorded from Japan.

Larvæ with dorsal tubercle on 4th segment.

90. *Egonocia perdix*.

Dashychira perdix Moore, Lep. Atk. p. 58, pl. 3. fig. 3 (1879); HAMPSON, Moths Ind. I. p. 450 (1892); l. c. IV. p. 460; WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 288.

Dashychira fasciatus Moore, Lep. Atk. p. 58.

Stauropus comatus Leech, Trans. Ent. Soc. Lond. 1898, p. 306.

?*Somera pryori* Leech, Trans. Ent. Soc. Lond. 1899, p. 216.

Local distribution. Nikko.

General distribution. Japan, China, India.

Time of appearance. June.

Identified from Moore's figure, but the specimens are much smaller measuring 35-45 mm. Considering that the larvæ feed on leaves of *Fagus* only the specimens now identified by me with *perdix* may possibly be separable from *perdix*.

Genus *Drymonia* Hübner (1822).

Palpi porrect, fringed with long woolly hair and not reaching beyond frons, 1st and 2nd joints almost equal in length, 3rd rather long and pointed or shorter than 2nd as in the preceding genus; proboscis vestigial; antennae in male pectinate to before the tip, in female "simple, setiform" (Grünberg); eyes naked. Legs hairy; foretibiae with the lobe not reaching the end, hindtibiae with two pairs of spurs. Forewing rather thinly scaled subcostal vein not hairy on underside; without tuft of scales on inner margin or it is feebly developed; vein 5 from middle of discocellulars; 6, 7, 8, 9 and 10 stalked or 6 from upper angle of cell; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ noctuiform, smooth, cylindrical, with 16 feet. Pupate under the ground and spin a rough cocoon.

Pupæ "with spinose anal end" (GRÜNBERG).

Geographical distribution. Palearctic, Oriental and Nearctic.

91. *Drymonia lineata*.

(Pl. XXII, fig. 21.)

Drymonia lineata Oberthür, Etud. Ent. V. p. 61, pl. 2. fig. 7 (1880); LEECH, Trans. Ent. Soc. Lond. 1898, p. 310; STAUDINGER, Cat. Lep. pal. p. 108 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 296, pl. 45 d (1912)

Phcosia octofasciata Matsumura, Zoku-senchiu-zukai. I. p. 54, pl. 9. fig. 9 (1909).

Local distribution. Shinano, Sapporo, Hakodate.

General distribution. Japan, Siberia.

Time of appearance. May, June.

92. *Drymonia trimacula*.

(Pl. XXX, fig. 10; Pl. XXXIX, fig. 1.)

Bombyx trimacula Esper, Schmett. III. p. 242, pl. 46. figs. 1-3 (1785); KIRBY, Cat. Lep. Het. p. 571 (1892); LEECH, Trans. Ent. Soc. Lond. 1898. p. 303; STAUDINGER, Cat. Lep. pal. p. 104 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 296, pl. 45 e (1912).

Notodonta trimacula var. *dodonides* Staudinger, Rom. Mém. III. p. 220 (1887).

?*Drymonia discoidalis* Matsumura, Zoku-senchiu-zukai. I. p. 72, pl. 11. fig. 6 (1909).

Local distribution. Tokyo, Shimashima.

General distribution. Japan, Siberia, Europe.

Time of appearance. June, July.

D. discoidalis Matsumura may possibly be a form of this species.

*93. *Drymonia basalis*.

Drymonia basalis Wileman et South, Entom. 1917, p. 28.

Local distribution. Gifu.

Habitat. Japan.

*94. *Drymonia chaonia*.

Bombix chaonia Hübner, Bomb. pl. 3. figs. 10, 11 (1800?); LEECH, Trans. Ent. Soc. Lond. 1898, p. 303; STAUDINGER, Cat. Lep. pal. p. 108 (1901); Grünberg, Seitz, Macrolep. II. p. 297, pl. 45e (1912).

Drymonia ruficornis Aurivillius, Nord Fjär. p. 72.

Local distribution. Gifu.

General distribution. Japan, Siberia, Europe.

Genus *Liparopsis* Hampson (1892).

I have not hitherto been able to examine the species of the genus.

95. *Liparopsis formosana*.

Liparopsis formosana Wileman, Entom. 1914, p. 323.

Local distribution. Kanshirei.

Habitat. Formosa.

Time of appearance. September.

Genus *Ochrostigma* Hübner (1827).

Palpi short and porrect or obliquely upturned, first two joints hairy, 3rd moderate or rather long and pointed; proboscis feebly developed; antennae in male pectinate to the tip or dentate and fasciculate, in female ciliated or "serrate and ciliated" (Grünberg); eyes naked; "ocelli small" (Grünberg).



Text-fig. 36. Foretibia of *O. japonica*, ♂. × 23.

Legs hairy; foretibiae with the lobe not reaching to the end, hindtibiae with two pairs of spurs. Forewing with a tuft of scales on inner margin, which is not strongly convexed; vein 5 from middle of discocellulars; 6, 7, 8, 9

and 10 stalked, or 6 from upper angle of cell; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell and connected with it by a short bar.

Larvæ slender, smooth, with or without hair. Pupate under the ground and spin a rough cocoon.

Pupæ "with short points at the anal end" (GRÜNBERG).

Geographical distribution. Palearctic.

Sect. I. Antennæ of male pectinate.

96. *Ochrostigma japonica*.

(Pl. XXIII, fig. 23; Pl. XXXIV, fig. 3; Text-fig. 36.)

Ochrostigma japonica Wileman, Trans. Ent. Soc. Lond. 1911, p. 285, pl. 30, fig. 25; GRÜNBERG, Seitz, Macrolep. II. p. 305 (1912).

Local distribution. Kaga, Yoshino, Nikko.

Habitat. Japan,

Time of appearance. June—September.

Sect. II. Antennæ of male serrate and fasciculate.

97. *Ochrostigma mantleyi*.

(Pl. XXV, fig. 2; Pl. XXVII, fig. 6; Pl. XXXI, fig. 3;

Pl. XXXIV, fig. 4.)

Drymonia mantleyi LEECH, P.Z.S. 1888, p. 639, pl. 22, fig. 2; Trans. Ent. Soc. Lond. 1898, p. 303; GRÜNBERG, Seitz, Macrolep. II. p. 296, pl. 45d (1912); NAGANO, Bull. Nawa Ent. Lab. I. p. 4, pl. 1, figs. 28–39, pl. 9, fig. 5, larva (1916).

Drymonia mantleyi var. *coreana* Nagano, Bull. Nawa Ent. Lab. I. p. 5, pl. 1, fig. 40 (1916).

Local distribution. Tokyo, Gifu, King-Kitao.

General distribution. Japan, Corea.

Time of appearance. October, November.

98. *Ochrostigma punctatella*.

(Pl. XXII, fig. 10.)

Orygia punctatella Motschulsky, Etud. Ent. 1860, p. 32; Wileman, Trans. Ent. Soc. Lond. 1911, p. 288.

Cnethodonta suzuki Takenouchi, Trans. Ent. Soc. Jap. II. p. 94 (1916).

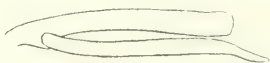
Local distribution. Tokyo, Takaosan, Nikko, Shinano, Yoshino, Tobetsu, Junsai Numa.

Habitat. Japan.

Time of appearance. April—July.

Genus *Ptilophora* Stephens (1828).

Palpi small, fringed with long woolly hair; proboscis vestigial; antennae in male plumose, in female "subserrated" (STEPHENS); eyes naked. Head and thorax densely woolly. Abdomen woolly with an anal tuft of long woolly hair. Legs hairy; foretibiae with the lobe reaching far beyond the end, hindtibiae with a pair of long slender spurs. Wings thinly scaled.



Text-fig. 37. Foretibia of *P. plumigera*, ♂. × 23.

Forewing with termen and inner margin fringed with long hair, the former strongly oblique, and forming an obtuse angle with the latter; vein 5 from middle of discocellulars; 6 from upper angle of cell; 7, 8, 9 and 10 stalked, 10 arising from before 7; 11 free; no areole. Hindwing with vein 5 from about middle of discocellulars; 6 and 7 stalked to near end of them; 8 running close along upper margin of cell.

Larvæ "slender, naked, Noctuid-like, without any tubercles; 16 feet" (GRÜNBERG).

Geographical distribution. Palearctic.

99. *Ptilophora plumigera*.

(Pl. XXII, fig. 22; Pl. XXXIX, fig. 5; Text-fig. 37.)

Bombyx plumigera Esper, Schmett. III. p. 254, pl. 50. figs. 6, 7 (1785); KIRBY, Cat. Lep. Het. p. 598 (1892); LEECH, Trans. Ent. Soc. Lond. 1898, p. 312; STAUDINGER, Cat. Lep. pal. p. 111 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 309, pl. 47g (1912).

Local distribution. Tokyo, Yokohama, Hokkaido.

General distribution. Japan, Siberia, Europe.

Time of appearance. January, October.

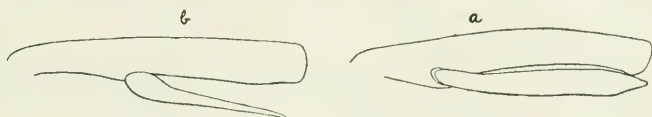
Genus **Ramesa** Walker (1855).

Pydna Walker (1855).

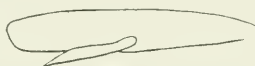
Ceira Walker (1855).

Bireta Walker (1864).

Palpi porrect or upturned, first two joints hairy, 2nd joint in male long, in female short, 3rd rather large and pointed; proboscis vestigial; antennæ in male pectinate or serrate and fasciculate to the tip, in female ciliated;



Text-fig. 38. Foretibiae of *R. straminea*, x23., a. ♂, b. ♀.



Text-fig. 39. Foretibia of *R. tosta*, ♀, x23.

eyes naked. Legs long and less hairy; foretibiae with the lobe reaching the end in male, hardly reaching in female, hindtibiae with two pairs of long spurs. Forewing without tuft of scales on inner margin; vein 5 from middle of discocellulars; 6 from upper angle of cell or shortly stalked with 7, 8 and 9; 10 from cell and anastomosing with 7, 8 and 9 or 8 and 9 to form a short or long areole. Hindwing with vein 5 from middle of discocellulars; 6 and 7 stalked or both free from upper angle of cell; 8 running close along upper margin of cell and connected with it by a bar.

Larvæ noctuiform, smooth and cylindrical; 16 feet. Pupate under the ground and spin a rough cocoon.

Pupæ with the wing reaching beyond middle; cremaster with 8 spines which are rolled up at their end.

Geographical distribution. Palearctic and Oriental.

The shape of the forewing is modified among the sexes, thus in the female the apex is more acute than in the male.

Sect. I. Forewing with the areole short, triangular; antennæ of male serrate and fasciculate.

100. *Ramesa tosta*.

(Pl. XXX, fig. 5; Pl. XXXVII, fig. 1; Text-fig. 39.)

Ramesa tosta Walker, Cat. V. p. 1017 (1855); BUTLER, Ill. Het. B.M. VI.

p. 13, pl. 104. fig. 5 (1886); HAMPSON, Moths Ind. I. p. 143 (1892).

Ramesa luridivitta Hampson, Ill. Het. B.M. IX. p. 59, pl. 160. fig. 12 (1893).

Local distribution. Yamakita.

General distribution. Japan, India.

Time of appearance. August.

A single female taken by ITO at Yamakita, Sagami, August 17, 1915.

The specimen has the veins 6 and 7 of hindwing arising from the upper angle of cell.

101. *Ramesa pallida*.

(Pl. XXVI, fig. 1; Pl. XXX, figs. 3, 4; Pl. XXXVII, fig. 6.)

Bireta pallida Butler, A.M.N.H. (4) XX. p. 473 (1877); Ill. Het. B.M. II.

p. 12, pl. 25. figs. 10, 11 (1878); HAMPSON, Moths Ind. I. p. 140 (1892);

LEECH, Trans. Ent. Soc. Lond. 1898, p. 301; GRÜNBERG, Seitz, Macrolep.

II. p. 316, pl. 56e (1912).

Local distribution. Tokyo, Yokohama, Shikoku, Hokkaido.

General distribution. Japan, China, India.

Time of appearance. May, June.

Larvæ feed on bamboo.

*102. *Ramesa sordita*.*Pydna sordita* Wileman, 1914. p. 267.

Local distribution. Rantaizan.

Habitat. Formosa.

Time of appearance. May.

*103. *Ramesa nebulosa*.*Pydna nebulosa* Wileman, Entom. 1914, p. 267.

Local distribution. Arizan.

Habitat. Formosa.

Time of appearance. August.

*104. *Ramesa kanshireiensis*.

Pydna kanshireiensis Wileman, Entom. 1914, p. 322.

Local distribution. Kanshirei.

Habitat. Formosa.

Time of appearance. May, July, September.

Sect. II. Forewing with the areole long; antennæ of male pectinate.

105. *Ramesa straminea*.

(Pl. XXIII, figs. 16, 17; Pl. XXX, figs. 1, 2;

Pl. XXXVII, fig. 5; Text-fig. 38.)

Ceira straminea Moore, A.M.N.H. (4) XX. p. 91 (1877); LEECH, Trans. Ent. Soc. Lond. 1898, p. 301; GRÜNBERG, Seitz, Macrolep. II. p. 316, pl. 47g (1912).

Local distribution. Tokyo, Yokohama, Nachi, Tanabe, Usuki, Hokkaido, Gensan.

General distribution. Japan, Corea, China.

Time of appearance. May, July, August.

The termen of the forewing in the male is evenly curved, while in the female it is almost straight, the ground colour of the forewing in the female is deeper than in the male and sometimes almost orange yellow.

The markings of forewing are subject to considerable variations. Among many specimens examined by me there are some which have much darker hindwing.

Larvæ feed on bamboo.

*106. *Ramesa southerlandii*.

Bireta southerlandii Holland, Trans. Amer. Ent. Soc. 1889, p. 73; LEECH, Trans. Ent. Soc. Lond. 1898, p. 301; GRÜNBERG, Seitz, Macrolep. II. p. 316 (1912).

I have hitherto been unable to examine the type, but this may possibly be referable to the preceding species by judging from its original description.

*107. *Ramesa inconspicua*.*Pydna inconspicua* Wileman, Entom. 1914, p. 267.

Local distribution. Arizona.

Habitat. Formosa.

Time of appearance. August.

*108. *Ramesa plumosa*.¹*Bireta plumosa* Leech, P.Z.S. 1888, p. 620, pl. 31. fig. 4; Trans. Ent. Soc.

Lond. 1898, p. 300; GRÜNBERG, Seitz, Macrolep. II. p. 316, pl. 49a (1912).

Local distribution. Oyama (Sagami).

Habitat. Japan.

*109. *Ramesa virgata*.*Pydna virgata* Wileman, Entom. 1914, p. 266.

Local distribution. Kanshirei.

Habitat. Formosa.

Antennæ in male ciliated.

*110. *Ramesa albifusa*.*Pydna albifusa* Wileman, Entom. 1910, p. 345.

Local distribution. Kanshirei.

Habitat. Formosa.

Time of appearance. April, August.

It is unknown to me which section this should belong to.

Genus *Pterostoma* Germer (1812).

Palpi abnormally long, fringed with long hair on the upper and under sides; 3rd joint long and pointed; proboscis feeble; antennæ pectinate to the tip; eyes naked. Legs hairy; foretibiæ with the lobe reaching the end, hindtibiæ with two pairs of spurs. Forewing with

Text-fig. 40. Foretibia of *P. sinicum*, ♂, ×23.

1. A male specimen taken by Kumasawa at Komono, Ise, July 21, 1919. Belongs to Sect. II.

termen more or less crenulate; a large tuft of scales at middle of inner margin and at anal angle; vein 5 from middle of discocellulars; 6 from just below upper angle of cell; 7, 8 and 9 stalked; 10 from cell and anastomosing with the stalk of 8 and 9 to form an areole. Hindwing with vein 5 from middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ "naked, noctuid like, moderately flat and relatively broad, with 16 feet, and 4 rows of granules commencing on the second thoracical segment, the tubercles of the two lateral rows smaller than those of the middle rows, there being an additional similar row on each side on a level with the spiracles. At rest the head is held horizontally forward, the frons being directed upward" (GRÜNBERG).

Pupæ "cylindrical with spinose anal end; in a cell in the ground lined with silk" (GRÜNBERG).

111. *Pterostoma sinicum*.

(Pl. XXII, fig. 4; Pl. XXXVI, fig. 4; Text-fig. 40.)

Pterostoma sinica MOORE, A.M.N.H. (4) XX. p. 91 (1877); STAUDINGER, Cat.

Lep. pal. p. 111 (1901); GRÜNBERG, Seitz, Macrolep. II. 309, pl. 47a, b (1912).

Pterostoma sinicum LEECH (nec MOORE), Trans. Ent. Soc. Lond. 1898, p. 314.

Pterostoma palpina var. *gigantea* STAUDINGER, Rom. Mém. VI. p. 363 (1892).

?*Pterostoma palpina* MATSUMURA (nec LINNÆUS), Cat. Ins. Jap. I. p. 38 (1905).

Local distribution. Tokyo, Yokohama, Oiwake, Kaga, Nagasaki, Hakodate.

General distribution. Japan, China, Siberia.

Time of appearance. April—August.

The species are very variable in colour, size and shape of forewing.

Prof. Matsumura states the presence of *P. palpina* in Japan, but it is very doubtful.

Genus *Gluphisia* Boisduval (1829).

Head and thorax woolly haired, abdomen also more or less woolly. Palpi minute fringed with long woolly hair, obliquely down-turned; proboscis

present; eyes sparsely hairy. Legs woolly; foretibiae with the lobe reaching



Text-fig. 41. Foretibia of *G. japonica*, ♂. x23.

the end, hindtibiae with a pair of spurs. Forewing rather broad with the anal angle not distinct; vein 5 from middle of discocellulars; 6 from upper angle of cell or stalked with

7, 8, 9 and 10; 11 free; no areole. Hindwing with vein 5 from middle of discocellulars which are inwardly angled at middle; veins 6 and 7 stalked to beyond middle; 8 running close along upper margin of cell.

Larvæ. "Body nectuiiform, tapering toward each end; smooth, entirely unarmed. Head rounded, smooth, with a black stripe on each side. Body with a subdorsal yellow line on each side of back, otherwise pale green, or with several dorsal pink patches" (Packard).

Cocoon. "Slight and thin, spun between leaves" (Packard).

Pupæ. "Flattened, oval, rounded obtusely at each end; cremaster obsolete, with no traces of spines" (Packard).

Geographical distribution. Palearctic and Nearctic.

112. *Gluphisia japonica*.

(Pl. XXIII, fig. 4; Pl. XXXV, fig. 5; Text-fig. 41.)

Gluphisia japonica Wileman. Trans. Ent. Soc. 1911, p. 289, pl. 30. fig. 12; Grünberg, Seitz, Macrolep. II. p. 295 (1912).

Local distribution. Sapporo, Hakodate, Tobetsu, Kyoto.

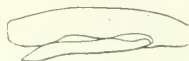
Habitat. Japan.

Time of appearance. June, August.

This may possibly be identical with *G. amurensis*, form of *G. crenata*.

Genus *Micromelalopha* Nagano (1916).

Palpi short, hairy, obliquely porrect, 3rd joint very small; proboscis feebly developed; antennæ pectinate to the tip in both sexes, the branches shorter in female; eyes hairy.



Text-fig. 42. Foretibia of *M. troglodyta*, ♂. x23.

Legs hairy, foretarsi and tibiae thickly haired as in *Melalopha*; foretibiae with the lobe not reaching

the end, hindtibiæ with a pair of spurs. Forewing without tuft of scales on inner margin; vein 5 absent; 6 from close upper angle of cell; 7, 8, and 10 free from cell; no areole. Hindwing with vein 5 absent; 6 and 7 stalked; 8 running close along upper margin of cell.

Larvæ "cylindrical, naked, each wart bearing single hair pupating in the ground" (Nagano).

Geographical distribution. Palearctic.

NAGANO states that vein 7 of the forewing is absent, but my examination proves that vein 5 is absent instead of vein 7.

113. *Micromelalopha troglodyta*.

(Pl. XXIII, fig. 25; Pl. XXIX, fig. 11; Pl. XXXVI, fig. 7;
Text-fig. 42.)

Pygæra troglodyta Græser, Berl. ent. Zeit. 1980, p. 12; STAUDINGER, Rom. Mém. VI. p. 372, pl. 5. fig. 7 (1892); Cat. Lep. pal. p. 112 (1901); WILEMAN, Trans. Ent. Soc. Lond. 1911, p. 297; GRÜNBERG, Seitz, Macrolep. II. p. 314, pl. 47f (1912).

Pygæra sieversi Staudinger, Rom. Mém. VI. p. 370, pl. 5. figs. 6a, b (1892); NAGANO, Bull. Nawa Ent. Lab. I. p. 10, pl. 3. figs. 33-43, pl. 9. fig. 11, larva (1916).

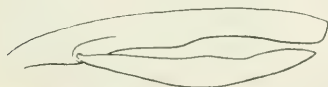
Local distribution. Yoshino, Gifu, Shimashima.

General distribution. Japan, Siberia.

Time of appearance. May, July, August.

Genus *Gonoclostera* Butler (1877).

Palpi obliquely porrect, 3rd joint moderate and pointed; proboscis feeble; antennæ pectinate to the tip in both sexes; eyes hairy. Thorax thickly haired with a slight crest at middle. Legs hairy, but not so in *Melalopha*; foretibiæ with the lobe reaching the end, hindtibiæ with two pairs of spurs. Forewing with termen excised below apex and angled between veins 4 and 5; no tuft of scales on



Text-fig. 43. Foretibia of *G. timoniles*, ♂. ×23.

inner margin; vein 5 from middle of discocellulars; 6 from upper angle of cell; 7, 8, 9 and 10 stalked; 11 free from cell; no areole. Hindwing with vein 5 absent; 6 and 7 stalked; 8 running close along upper margin of cell and connected with it by a bar.

Larvæ cylindrical, slightly humped at middle, sparsely haired, with 16 feet. Geographical distribution. Palearctic.

114. *Gonoclostera timonides*.

(Pl. XXII, fig. 18; Pl. XXXI, fig. 5; Pl. XXXVI, fig. 5;

Text-fig. 43.)

Pygerra timonides Bremer, Lep. Ost-Sib. p. 45, pl. 5, fig. 3 (1864); OBERTHÜR, Etud. Ent. X. p. 13, pl. 2, fig. 2 (1884); LEECH, Trans. Ent. Soc. Lond. 1898, p. 318; STAUDINGER, Cat. Lep. pal. p. 112 (1901); GRÜNBERG, Seitz, Macrolep. II. p. 313, pl. 47f (1912); NAGANO, Bull. Nawa Ent. Lab. I. p. 9, pl. 3, figs. 22-32, pl. 9, fig. 14, larva (1916).

Pygerra timoniorum Bremer, l.c. pl. 5, fig. 3.

Gonoclostera latipennis Butler, A.M.N.H. (4) XX. p. 476 (1877).

Pygerra trimonides Matsumura (nec Bremer), Cat. Ins. Jap. I. p. 39 (1905).

Local distribution. Tokyo, Kaga, Gifu, Matsumoto, Yokohama, Nikko, Hakodate.

General distribution. Japan, China, Siberia.

Time of appearance. April—September.

Genus *Densitas* n. gn.

Pulpi obliquely upturned to middle of frons, first two joint fringed with long woolly hair, 3rd minute; proboscis vestigial; antennæ in male pectinate to the tip; eyes naked. Head, thorax and abdomen woolly haired. Legs woolly; foretibiae with the lobe reaching the end, hindtibiae with a pair of spurs. Forewing without tuft of scales on inner margin; vein 5 from middle of discocellulars; 6 from upper angle of cell; 7, 8, 9 and 10 stalked; 11 free from cell; no areole. Hindwing with vein 5 from above middle of discocellulars; 6 and 7 stalked; 8 running close along upper margin of cell; frenulum absent.

Early stages unknown.

Type. *D. permagnum*.

Geographical distribution. Palearctic.

115. *Densitas permagnum*.

(Pl. XXIII, fig. 15; Pl. XXXVIII, fig. 8.)

Drymonia permagna Butler, Trans. Ent. Soc. Lond. 1881, p. 20; LEECH, Trans. Ent. Soc. Lond. 1898, p. 304; GRÜNBERG, Seitz, Macrolep. II. 297 (1912).

Zugra ziozankeana Matsumura, Zoku-senchiu-zukai. I. p. 65, pl. 10. fig. 19 (1909).

Local distribution. Tokyo, Shinano, Ziozankei.

Habitat. Japan.

Time of appearance. August.

POSTSCRIPT.

When I was going to put my manuscript to press, Prof. MATSUMURA published his paper on the Japanese Notodontidæ in the Zoological Magazine, Vol. XXXI. No. 365 (in Japanese). He recognized the following new and unrecorded species:

Dudusa sphingiformis Moore.

Pheosia dictceoides Esper.

Somera viridifusca Walker.

Pygopteryx suava Staudinger.

Ramesa tosta Walker.

Drymonia daisenensis Matsumura.

Hypodonta lignea Matsumura.

Shachia subrosea Matsumura.

Epinotodonta fumosa Matsumura.

Hyperacsehra suzukiana Matsumura.

H. serrata Matsumura.

H. angustipennis Matsumura.

Nikko.

Sapporo, Shinano.

Formosa.

Sapporo Gifu.

Izu.

Hoki.

Sapporo, Nikko.

Sapporo.

Shinano.

Kyoto.

Kyoto.

Kyoto.

<i>H. nigricollalis</i> Matsumura.	Sapporo.
<i>Lophopteryx kuwayamae</i> Matsumura.	Hakodate, Sapporo.
<i>L. jezoensis</i> Matsumura.	Sapporo.
<i>Phalera angustipennis</i> Matsumura.	Tokyo.
<i>P. takasagansis</i> Matsumura.	Harima.
<i>P. jezoensis</i> Matsumura.	Sapporo, Kyoto.

On account of the earlier publishing of his paper the names adopted by me in the present paper should be synonymous as follows :

Yazaucia japonica = *Epinotodonta fumosa* Matsumura.

Hupodonta absoleta = *Hupodonta lignea* Matsumura.

His new species—*Shachia subrosca*, *Phalera angustipennis*—should be synonymous as follows :

Shachia subrosca Matsumura = *Microhoplitis circumscripta* Butler.

Phalera angustipennis Matsumura = *Phalera fuscescens* Butler.

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EXPLANATION OF PLATES.

PLATE XXII.

- Fig. 1. *Hyperaschra taicana* n. sp., ♂.
 Fig. 2. *Phalera assimilis* BREM. et GREY, ♀.
 Fig. 3. *Eulampsonia cristata* BUTL., ♂.
 Fig. 4. *Pterostoma sinicum* MOORE, ♂.
 Fig. 5. *Uropygia meticulodina* OBERTH., ♂.
 Fig. 6. *Macrurocampa signata* BUTL., ♂.
 Fig. 7. *Hoplites milhauseri* FABR., ♀.
 Fig. 8. *Microhoplitis circumscripta* BUTL., ♂.
 Fig. 9. *Brachionycoides atrovittatum* BREM., ♀.
 Fig. 10. *Ochrostigma punctatella* MOTSCH., ♀.
 Fig. 11. *Ithodonta obsoleta* n. sp., ♂.
 Fig. 12. *Lophocosma atriplaga* STAUD., ♂.
 Fig. 13. *Cerura erminea mencianni* MOORE, ♀.
 Fig. 14. *Tarsolepis sommeri* HÜBN., ♂.
 Fig. 15. *Notodonta stigmatica* GRÜNBERG, ♂.
 Fig. 16. *Urodonta virilimicta* BREM., ♀.
 Fig. 17. *Lophodonta græseri* STAUD., ♂.
 Fig. 18. *Gonoclostera timoniles* BREM., ♂.
 Fig. 19. *Lophontesia pryri* BUTL., ♂.
 Fig. 20. *L. cuculus* STAUD., ♂.
 Fig. 21. *Drymonia lineata* OBERTH., ♂.
 Fig. 22. *Ptilophora plumigera* ESP., ♂.
 Fig. 23. *Hyperaschra biloba* OBERTH., ♀.
 Fig. 24. *H. tenebrosa* MOORE?, ♂.

PLATE XXIII.

- Fig. 1. *Pheosia dictyoides* ESP., ♂.
 Fig. 2. *Stauropus basalis* MOORE, ♀.
 Fig. 3. *Cnethodonta grisescens* STAUD., ♀.
 Fig. 4. *Glaphysia japonica* WILEMAN, ♂.
 Fig. 5. *Egonocia cyanea* LERCH, ♂.
 Fig. 6. *E. nichiensis* n. sp., ♂.
 Fig. 7. *E. formosana* n. sp., ♂.
 Fig. 8. *Fentonia ocypte* BREM., ♂.
 Fig. 9. *Leucodonta bicoloria* SCHIFF., ♂.
 Fig. 10. *Gangaridopsis citrini* WILEMAN, ♂.
 Fig. 11. *Nerice davidii* OBERTH., ♂.

- Fig. 12. *Microphalera grisea* BUTL., ♂.
 Fig. 13. *Allo donta leucodera* STAUD., ♂.
 Fig. 14. *Yazuia japonica* n. sp., ♂.
 Fig. 15. *Densitas permagnus* BUTL., ♂.
 Fig. 16. *Ramesa straminea* MOORE, ♂.
 Fig. 17. Do., ♀.
 Fig. 18. *Spatialia cinnamomea* LEECH, ♂.
 Fig. 19. *S. plusiotis* OBERTH?, ♂.
 Fig. 20. *S. dives* OBERTH., ♂.
 Fig. 21. *Lophopteryx capucina giraffina* HÜBN., ♂.
 Fig. 22. *L. saturata hoegei* GRÆS., ♂.
 Fig. 23. *Ochrostigma japonica* WILEMAN, ♂.
 Fig. 24. *Wilemanus videntatus* WILEMAN, ♀.
 Fig. 25. *Micromelalopha troglodyta* GRÆS., ♂.
 Fig. 26. *Himeropteryx miraculosa* STAUD., ♂.
 Fig. 27. Larva of *Fentonia ocypte*.
 Fig. 28. Cocoon of do.
 Fig. 29. Pupa of do.
 Fig. 30. Larva of *Egonocia cyanea*. (Yamada del.).
 Fig. 31. Cocoon of *E. cyanea*.
 Fig. 32. Pupa of do.
 Fig. 33. Larva of *Ilupodonta pulcherrima corticalis*, about ×2. (Yano del.).

PLATE XXIV.

- Fig. 1. A part of antenna of *Phalera flarescens*, ♂. ×25.
 Fig. 2. Do. of do., ♀. ×25.
 Fig. 3. Do. of *Phalera assimilis*, ♂. ×25.
 Fig. 4. Do. of do., ♀. ×25.
 Fig. 5. Do. of *Urodonta viridimixta*, ♀. ×37.
 Fig. 6. Do. of *Spatialia cinnamomea*, ♂. ×37.
 Fig. 7. Do. of *S. plusiotis*?, ♂. ×37.
 Fig. 8. Do. of *Spatialia darriesi*, ♂. ×37.
 Fig. 9. Do. of *S. dives*, ♂. ×37.
 Fig. 10. Hind-leg of *Phalera assimilis*, ♀. ×5.
 Fig. 11. Do. of *Spatialia cinnamomea*, ♂. ×8.
 Fig. 12. Do. of *darriesi*, ♂. ×7.
 Fig. 13. Do. *S. plusiotis*?, ♂. ×7.

PLATE XXV.

- Fig. 1. A part of antenna of *Lophopteryx capucina giraffina*, ♂. ×37.
 Fig. 2. Do. of *Ochrostigma manleyi*, ♂. ×38.
 Fig. 3. Do. of *Brachionycoides atrovittatus*, ♂. ×38.

- Fig. 4. Do. of *Yazawaia japonica*, ♂. × 37.
 Fig. 5. Do. of *Lophopteryx ladislai*, ♂. × 37.
 Fig. 6. Do. of *L. admirabilis*, ♂. × 37.
 Fig. 7. Do. of *Allodonta leucodera*, ♂. × 37.
 Fig. 8. Hind-leg of *Yazawaia japonica*, ♂. × 7.
 Fig. 9. Do. of *Lophopteryx capucina giraffina*, ♂. × 7.
 Fig. 10. Do. of *Nerice bipartita*, ♂. × 6.

PLATE XXVI.

- Fig. 1. A part of antenna of *Ramesa pallida*, ♂. × 37.
 Fig. 2. Do. of *Hyperaschra tenebrosa*?, ♂. × 37.
 Fig. 3. Do. of *Macrurocampa sigmata*, ♂. × 37.
 Fig. 4. Do. of *Lophodonta graseri*, ♂. × 37.
 Fig. 5. Do. of *Hyperaschra basilinea*, ♂. × 37.
 Fig. 6. Do. of *Macrurocampa variegata*, ♂. × 37.
 Fig. 7. Do. of *Lophodonta monetaria*, ♂. × 38.
 Fig. 8. Hind-leg of *Hyperaschra tenebrosa*, ♂. × 6.
 Fig. 9. Do. of *Lophopteryx admirabilis*, ♂. × 7.
 Fig. 10. Do. of *Urodonta viridimixta*, ♂. × 7.

PLATE XXVII.

- Fig. 1. A part of *Notodonta dembowskii*, ♂. × 37.
 Fig. 2. Do. of *Fentonia ocypte*, ♂. × 37.
 Fig. 3. Do. of *Wilemanus bidentatus*, ♂. × 38.
 Fig. 4. Hind-leg of *Macrurocampa variegata*, ♂. × 5.
 Fig. 5. Do. of *Microphalera grisea*, ♂. × 5.
 Fig. 6. Do. of *Ochrostigma manleyi*, ♂. × 7.
 Fig. 7. Do. of *Stauropus basalis*, ♂. × 7.
 Fig. 8. Do. of *S. fagi*, ♂. × 6.
 Fig. 9. Do. of *Lophocosma atriplaga*, ♂. × 5.
 Fig. 10. Do. of *Tarsolepis sommeri*, ♂. × 6.
 Fig. 11. Do. of *Hoplites milhauseri*, ♂. × 6.
 Fig. 12. Do. of *Lophontesia pryeri*, ♂. × 6.
 Fig. 13. Do. of *Erachionycoides atrovittatum*, ♂. × 6.
 Fig. 14. Palpus of *Hoplites milhauseri*, ♂. × 23.
 Fig. 15. Do. of *Microhoplitis circumscripta*, ♂. × 37.
 Fig. 16. Do. of *Lophontesia pryeri*, ♂. × 37.

PLATE XXVIII.

- Fig. 1. Palpus of *Tarsolepis sommeri*, ♂. × 25.
 Fig. 2. Do. of *Phalera assimilis*, ♂. × 33.

- Fig. 3. Do. of *P. flavescens*, ♂. × 38.
 Fig. 4. Do. of *Spatialia cinnamomea*, ♂. × 39.
 Fig. 5. Do. of *S. ornata*, ♂. × 25.
 Fig. 6. Do. of *S. plusiotis*?, ♂. × 37.
 Fig. 7. Do. of *Cnethodonta grisescens*, ♂. × 37.
 Fig. 8. Do. of *Spatialia darriesi*, ♂. × 25.
 Fig. 9. Do. of *S. dives*, ♂. × 38.
 Fig. 10. Do. of *Stawropus basalis*, ♂. × 37.
 Fig. 11. Do. of *Lophocosma atriplaga*, ♂. × 23.

PLATE XXIX.

- Fig. 1. Palpus of *Allodonta leucodera*, ♂. × 37.
 Fig. 2. Do. of *Yazawaia japonica*, ♂. × 37.
 Fig. 3. Do. of *Fentonia ocypte*, ♂. × 37.
 Fig. 4. Do. of *Hyperaschra tenelrosa*?, ♂. × 37.
 Fig. 5. Do. of *H. biloba*, ♂. × 38.
 Fig. 6. Do. of *Brachionyoiles atrovittatum*, ♂. × 38.
 Fig. 7. Do. of *Urodonta viridimixta*, ♂. × 23.
 Fig. 8. Do. of *Cerura vinula*, ♂. × 38.
 Fig. 9. Do. of *Pheosia dictyoides*, ♂. × 38.
 Fig. 10. Palpus of *Microphalera grisea*, ♂. × 37.
 Fig. 11. Do. of *Micromelalopha troglodyta*, ♂. × 38.
 Fig. 12. Do. of *Melalopha anachoreta*, ♂. × 38.

PLATE XXX.

- Fig. 1. Palpus of *Ramesa straminea*, ♂. × 38.
 Fig. 2. Do. of do., ♀. × 38.
 Fig. 3. Do. of *Ramesa pallida*, ♂. × 38.
 Fig. 4. Do. of do., ♀. × 38.
 Fig. 5. Do. of *Ramesa tosta*, ♀. × 38.
 Fig. 6. Do. of *Hypodonta pulcherrima corticalis*, ♂. × 23.
 Fig. 7. Do. of *Lophodonta græveri*, ♂. × 38.
 Fig. 8. Do. of *Macrurocampa variegata*, ♂. × 37.
 Fig. 9. Do. of *Notodonta dembowskii*, ♂. × 37.
 Fig. 10. Do. of *Drymonia trimacula dodonides*, ♂. × 38.
 Fig. 11. Do. of *Wilemanus bidentatus*, ♂. × 38.
 Fig. 12. Do. of *Nerice bipartita*, ♂. × 23.

PLATE XXXI.

- Fig. 1. Palpus of *Lophopteryx capucina giraffina*, ♂. × 23.

- Fig. 2. Do. of *L. admirabilis*, ♂. × 37.
 Fig. 3. Do. of *Ochrostigma manleyi*, ♂. × 23.
 Fig. 4. Do. of *Egonocia cyanea*, ♂. × 23.
 Fig. 5. Do. of *Gonoclostera timonides*, ♂. × 37.
 Fig. 6. Do. of *Euhampsonia cristata*, ♂. × 23.
 Fig. 7. Do. of *Macrurocampa signata*, ♂. × 23.
 Fig. 8. Wings of *Phalera flavesceus*, ♂. × 3. *a*, head of do., ♂.
 Fig. 9. Do. of *Tarsolepis sommeri*, ♂. × 2. *a*, head of do., ♂. *b*, lateral view of do., ♂.

PLATE XXXII.

- Fig. 1. Wings of *Stauropus fagi*, ♂. × 2. *a*, head of do., ♂.
 Fig. 2. Do. of *S. basalis*, ♂. × 3. *a*, head of do., ♂. *b*, a part of forewing of a specimen of do, showing the vein 1a not connected with 1b to form a fork at base. × 23.
 Fig. 3. Do. of *S. cinnamomea*, ♀. × 4. *a*, head of do., ♂.
 Fig. 4. Do. of *S. ornata*, ♂. × 4. *a*, head of do., ♂.
 Fig. 5. Do. of *S. dorrieri*, ♂. × 3. *a*, head of do., ♂.

PLATE XXXIII.

- Fig. 1. Wings of *Brachionycoides atrovittatum*, ♀. × 2. *a*, head of do., ♂.
 Fig. 2. Do. of *Uropygia meticulodina*, ♀. × 2. *a*, head of do., ♂.
 Fig. 3. Wings of *Lophocosma atriplaga*, ♂. × 2. *a*, head of do., ♂.
 Fig. 4. Do. of *Microphalera grisea*, ♂. × 3.
 Fig. 5. Do. of *Leucodonta bicoloria*, ♂. × 3. *a*, head of do., ♂.
 Fig. 6. Do. of *Lophontesia pryori*, ♂. × 4. *a*, head of do., ♂.
 Fig. 7. Do. of *Fentonia ocypte*, ♂. × 3. *a*, *b*, heads of do., ♂.
 Fig. 8. Do. of *Wilemanus bidentatus*, ♂. × 3. *a*, head of do., ♂.

PLATE XXXIV.

- Fig. 1. Wings of *Spatalia dives*, ♂. × 3. *a*, head of do., ♂.
 Fig. 2. Do. of *S. phasiotis*?, ♂. × 3. *a*, head of do., ♂.
 Fig. 3. Do. of *Ochrostigma japonica*, ♂. × 4. *a*, head of do., ♂.
 Fig. 4. Do. of *O. manleyi*, ♂. × 3. *a*, head of do., ♂.
 Fig. 5. Do. of *Cnethodonta griseusceus*, ♂. × 3. *a*, head of do., ♂.
 Fig. 6. Do. of *Microhoplitis circumscripta*, ♂. × 3. *a*, head of do., ♂.
 Fig. 7. Do. of *Cerura vinula*, ♂. × 2.
 Fig. 8. Hind-wing of *C. lanigera*, ♂. × 4. *a*, head of do., ♂.

PLATE XXXV.

- Fig. 1. Wings of *Euhampsonia cristata*, ♂. × 2. *a*, head of do., ♂.
 Fig. 2. Do. of *Urodonta viridimixta*, ♂. × 2. *a*, head of do., ♂.
 Fig. 3. Do. of *Alloclonta leucodera*, ♂. × 3. *a*, head of do., ♂.

- Fig. 4. Do. of *Yazuzata japonica*, ♂.×3. a, head of do., ♂.
 Fig. 5. Do. *Gluphisia japonica*, ♂.×3. a, head of do., ♂.
 Fig. 6. Do. of *Macrurocampa sigmata*, ♂.×3. a, head of do., ♂.

PLATE XXXVI.

- Fig. 1. Wings of *Hoplites milhauseri*, ♂.×3. a, head of do., ♂.
 Fig. 2. Do. of *Lophodonta monetaria*, ♂.×3. a, head of do., ♂.
 Fig. 3. Do. of *Gangaridopsis citrina*, ♂.×2. a, head of do., ♂.
 Fig. 4. Do. of *Pterostoma sinicum*, ♂.×2. a, head of do., ♂.
 Fig. 5. Do. of *Gonoclostera timonides*, ♂.×4. a, head of do., ♂.
 Fig. 6. Do. of *Hyperaschra biloba*, ♂.×3. a, head of do., ♂.
 Fig. 7. Do. of *Micromelalopha troglodyta*, ♀.×4. a, head of do., ♂.
 Fig. 8. Do. of *Lophopteryx lulistai*, ♂.×3. a, head of do., ♂.

PLATE XXXVII.

- Fig. 1. Wings of *Ramesa tosta*, ♀.×3. a, head of do., ♀.
 Fig. 2. Do. of *Lophopteryx capucina giraffina*, ♂.×3. a, head of do., ♂.
 Fig. 3. Do. of *Egonocia nachiensis*, ♂.×3. a, head of do., ♂.
 Fig. 4. Do. of *Egonocia cyanea*, ♂.×3. a, head of do., ♂.
 Fig. 5. Wings of *Ramesa straminea*, ♂.×3. a, head of do., ♂.
 Fig. 6. Do. of *R. pallida*, ♂.×3. a, head of do., ♂.

PLATE XXXVIII.

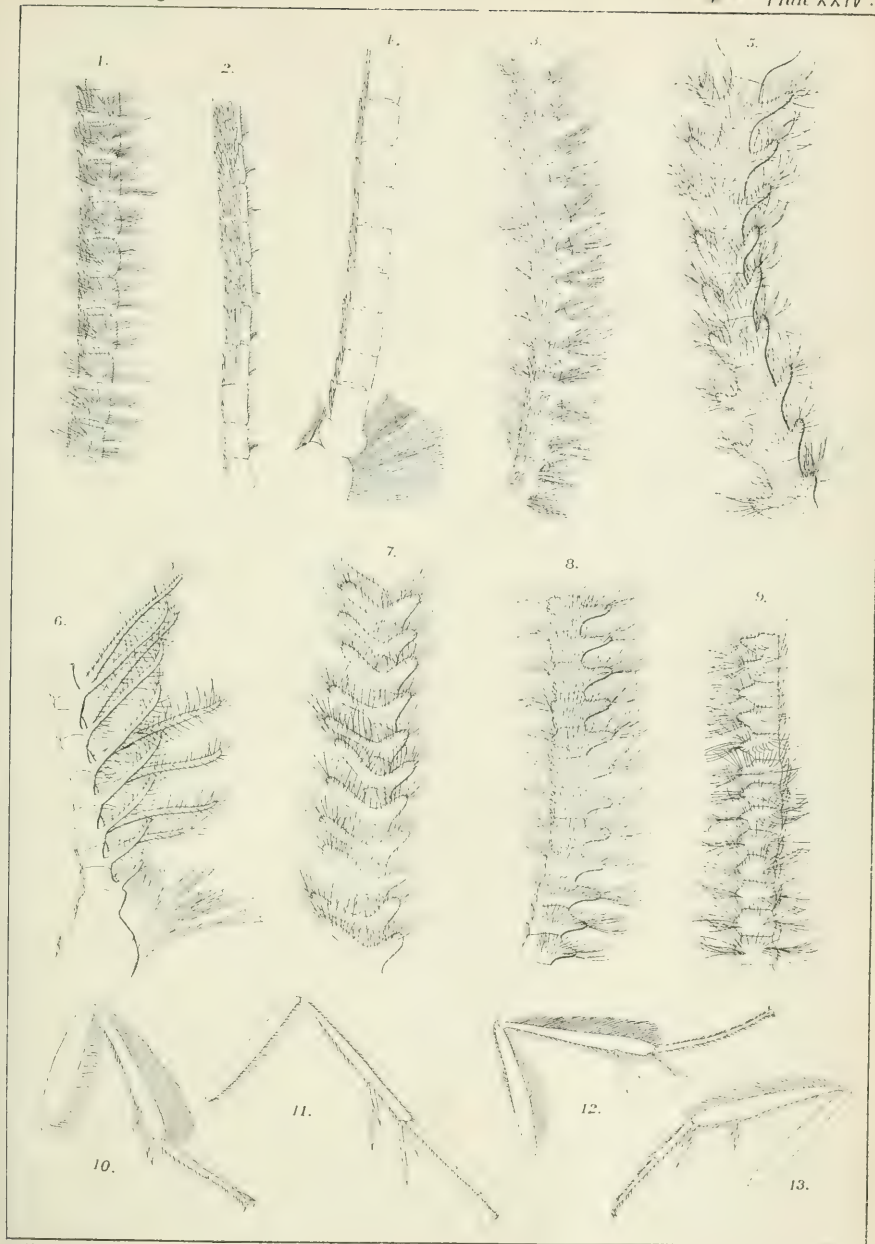
- Fig. 1. Wings of *Pheosia dictavoides*, ♂.×3. a, head of do., ♂.
 Fig. 2. Do. of *Hyperaschra tenebrosa?* ♂.×3. a, head of do., ♂.
 Fig. 3. Do. of *Macrurocampa variegata*, ♂.×3. a, head of do., ♂.
 Fig. 4. Do. of *Hypodonta pulcherrima corticalis*, ♂.×4. a, head of do., ♂.
 Fig. 5. Do. of *Melalopha anastomosis*, ♂.×4. a, head of do., ♂.
 Fig. 6. Do. of *M. anachoreta*, ♂.×4. a, head of do., ♂.
 Fig. 7. Do. of *Verice davidii*, ♂.×2. a, head of do., ♂.
 Fig. 8. Do. of *Densitas permagnum*, ♂.×2.

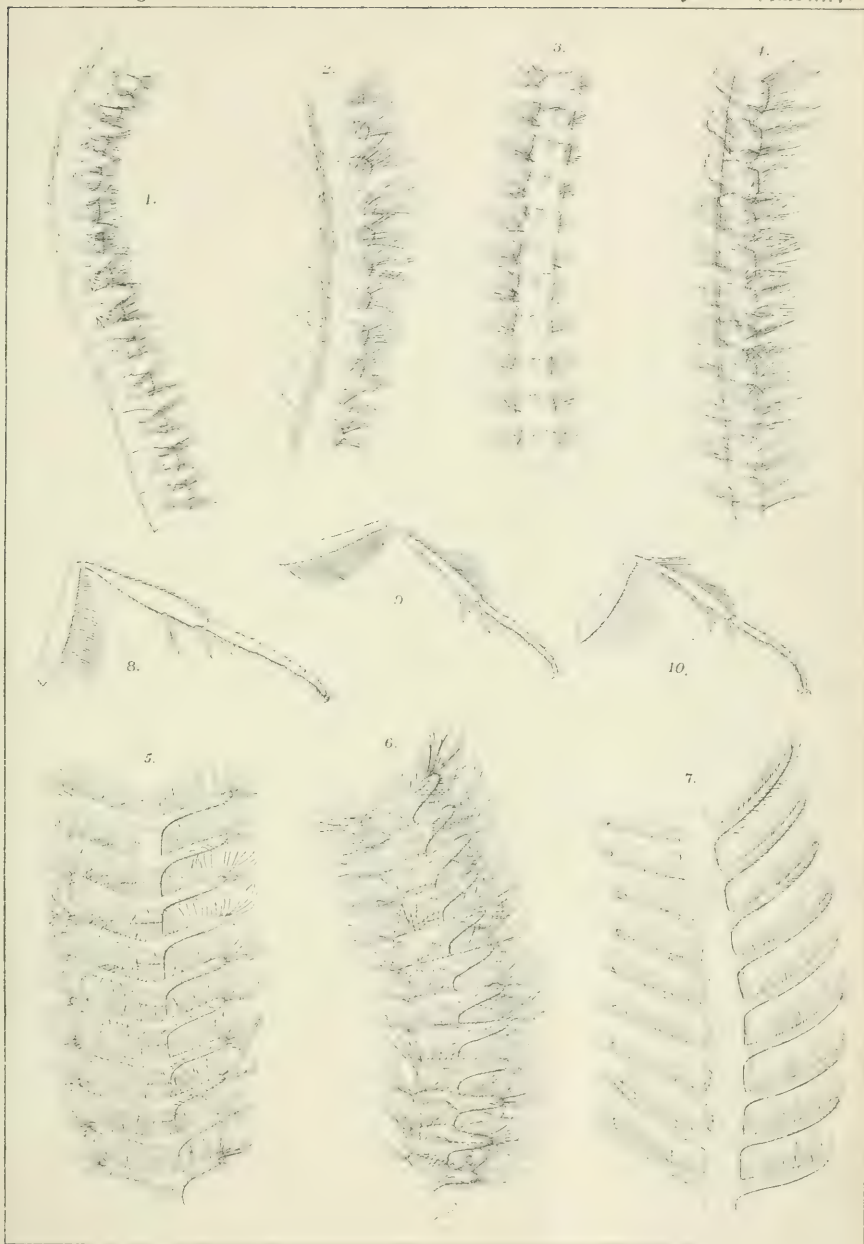
PLATE XXXIX.

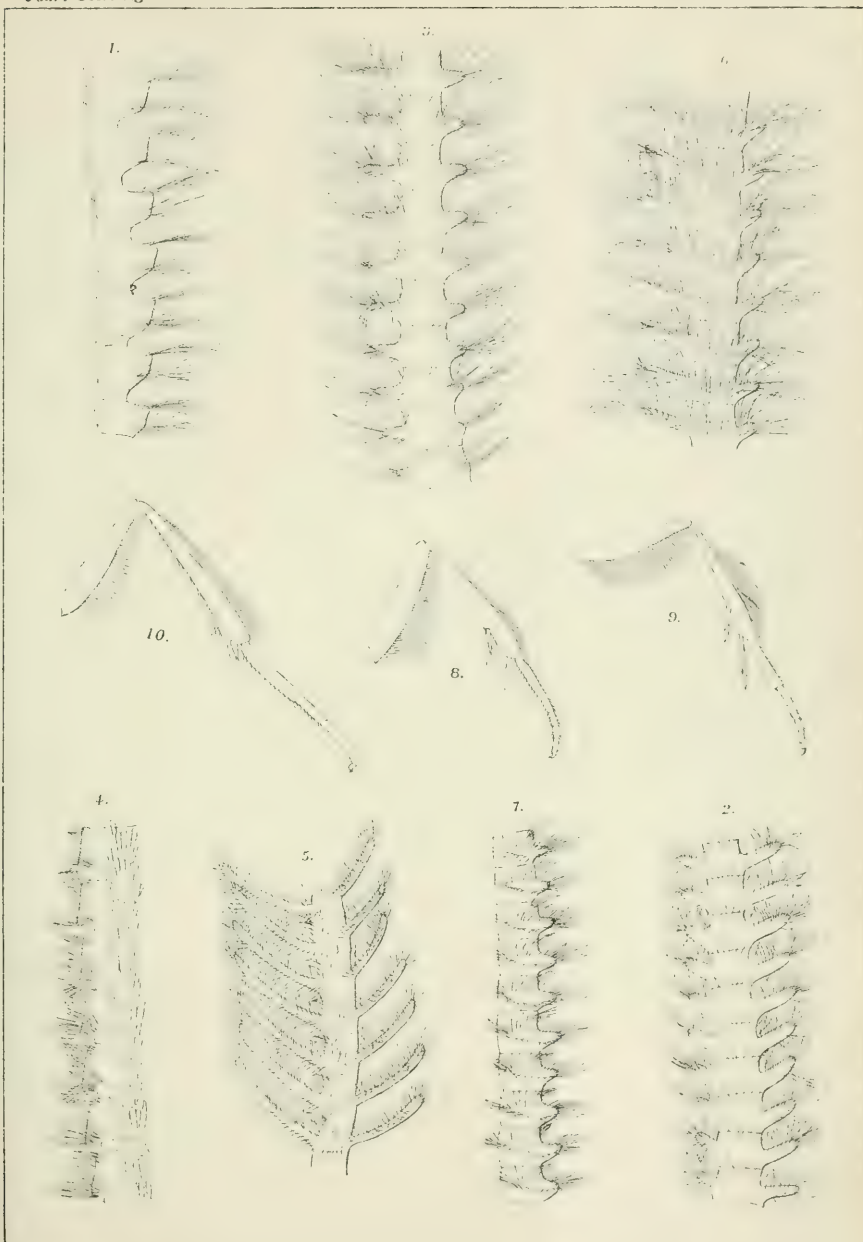
- Fig. 1. Wings of *Drymonia trimacula dodonides*, ♂.×3. a, head of do., ♂.
 Fig. 2. Do. of *Notodonta demboeskii*, ♂.×3. a, head of do., ♂.
 Fig. 3. Do. of *Hyperaschra basilinea*, ♂.×3. a, head of do., ♂.
 Fig. 4. Do. of *Macrurocampa delia*, ♂.×3. a, head of do., ♂.
 Fig. 5. Do. of *Ptilophora plumigera*, ♂.×4. a, head of do., ♂. b, Hind-leg of do., ♂.×7.
 Fig. 6. Do. of *Himeropteryx miraculosa*, ♂.×3. a, head of do., ♂.
 Fig. 7. Do. of *Phalera combusta*, ♂.×2.5. a, head of do., ♂.
 Fig. 8. Hind-leg of *Allodonta leucodera*, ♂.×6.

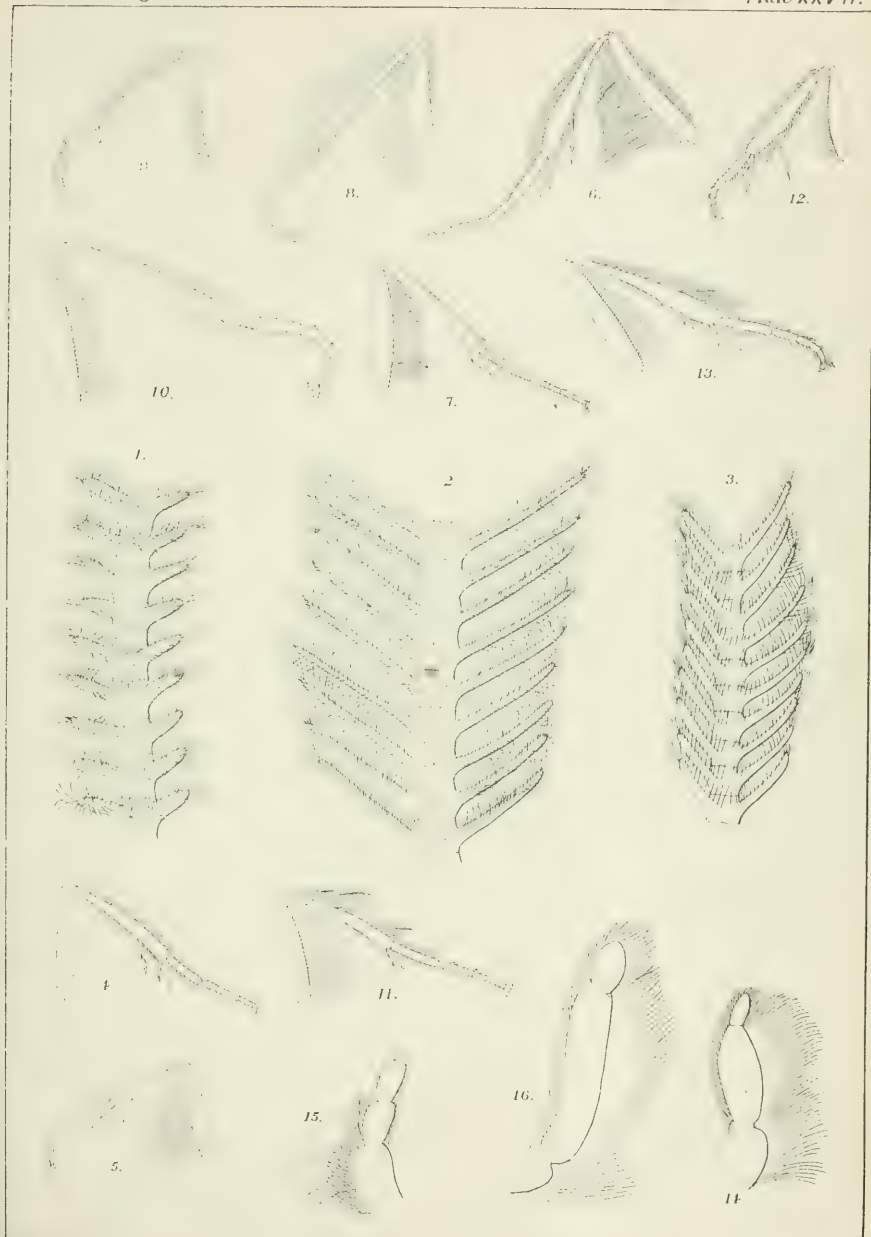


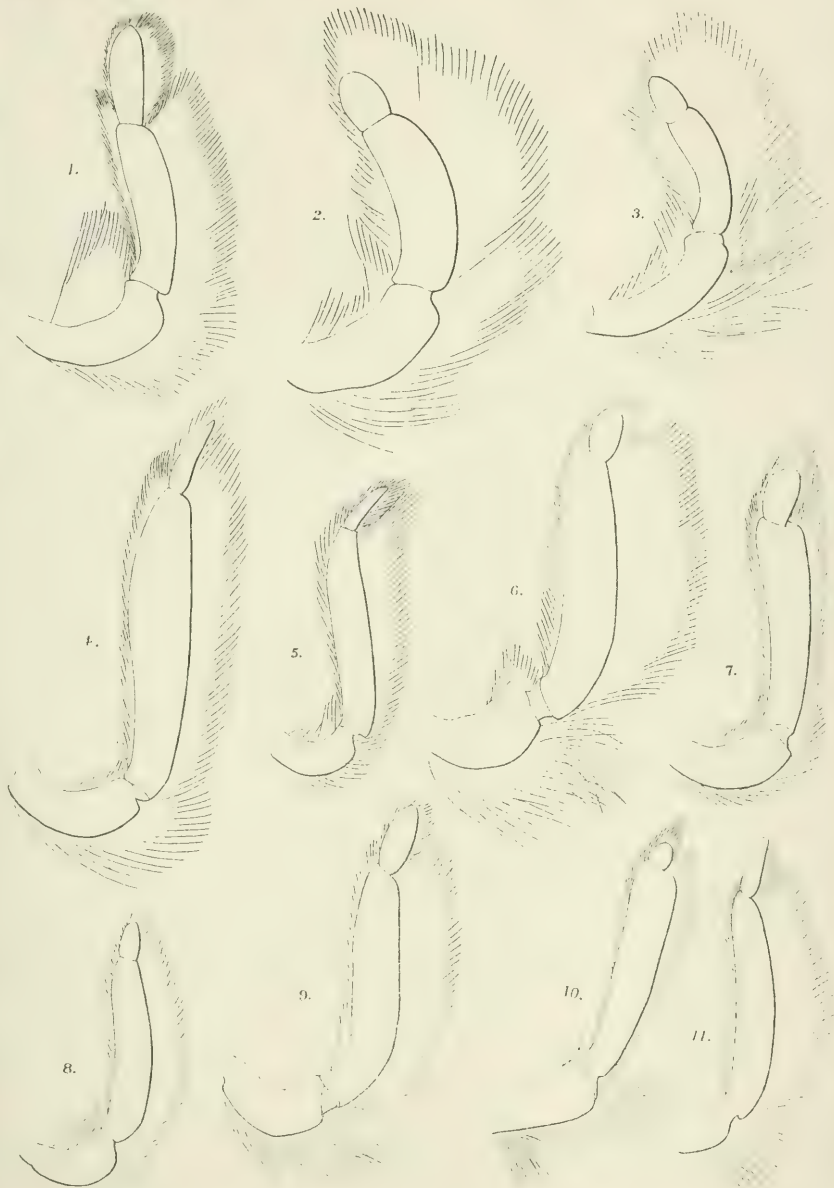


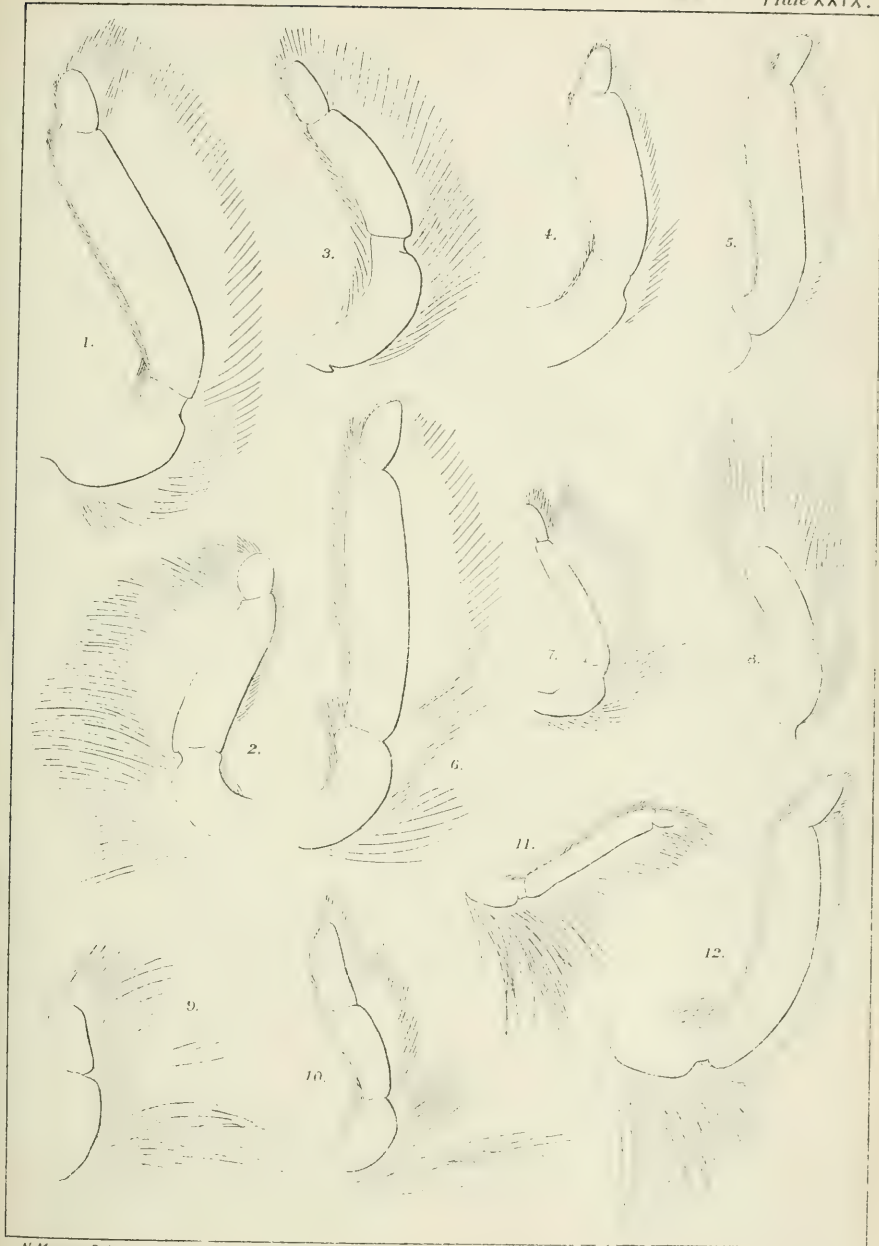


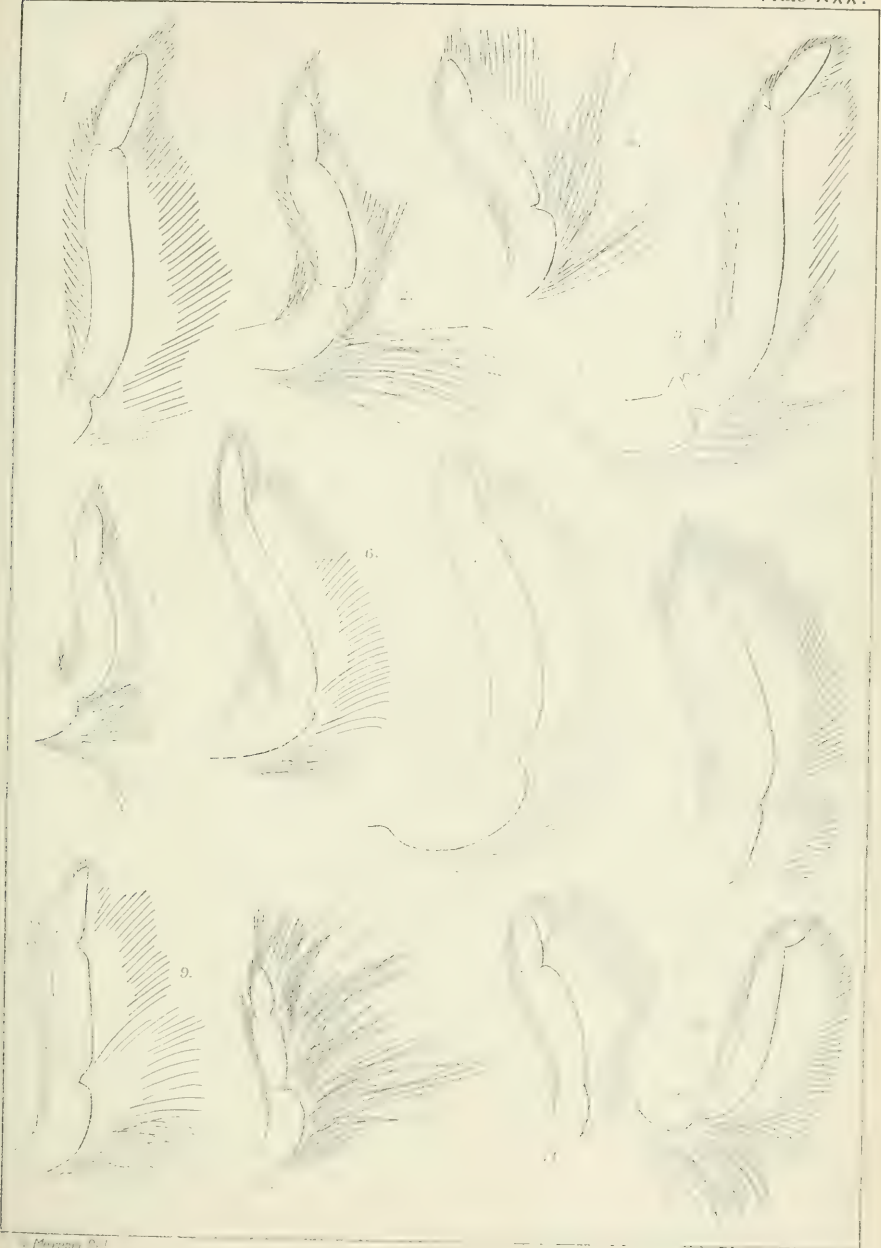


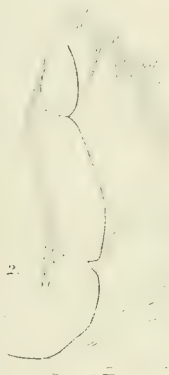
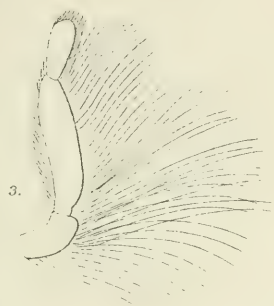


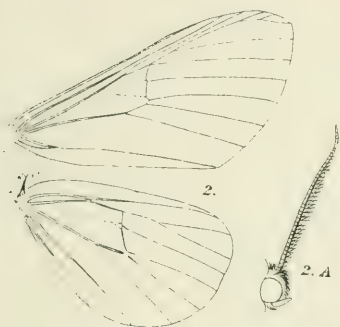
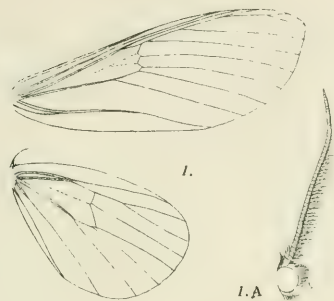


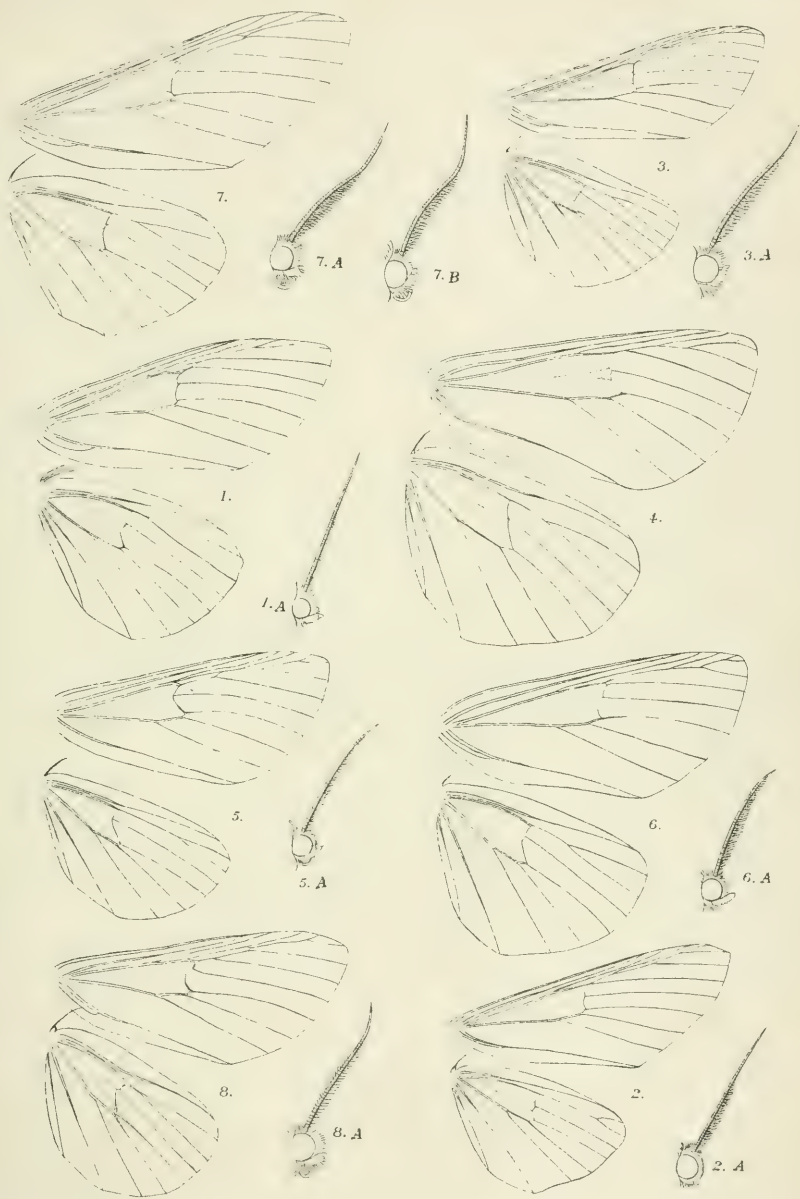


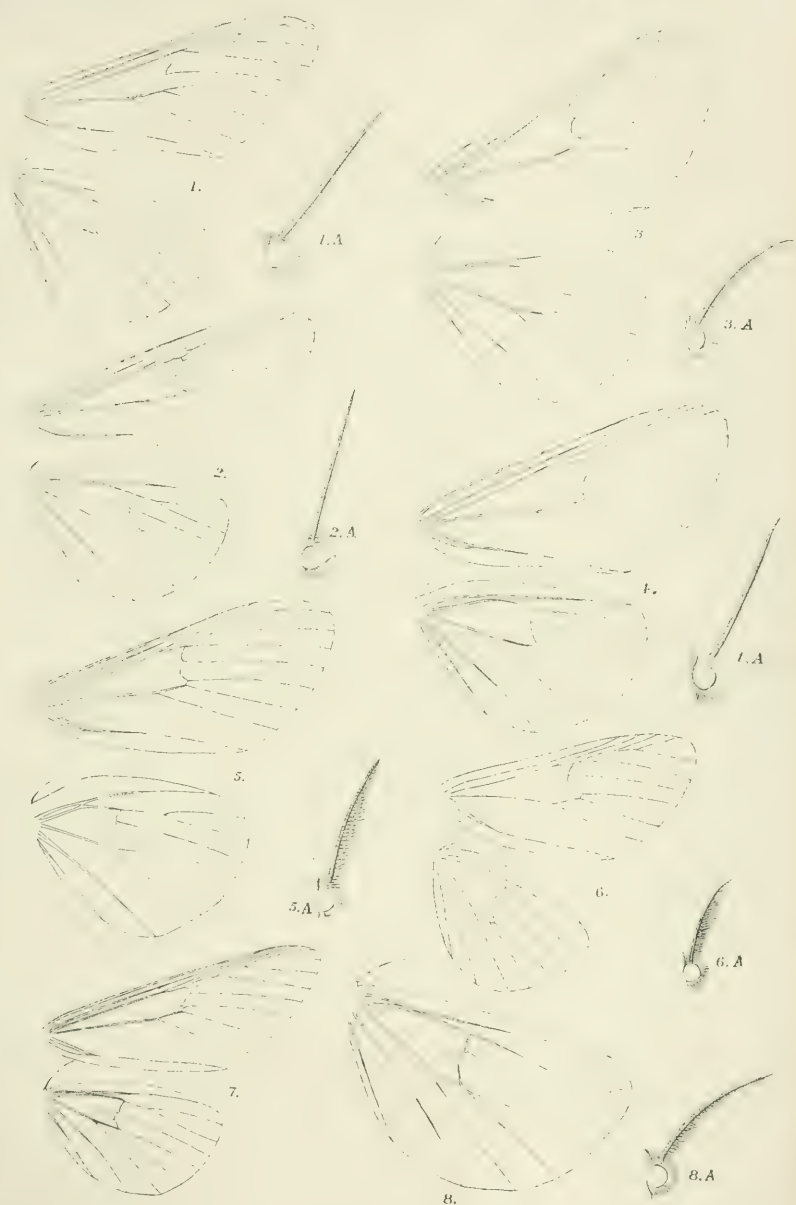


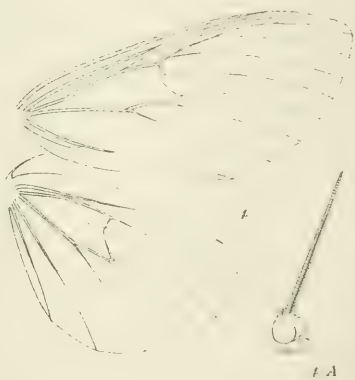
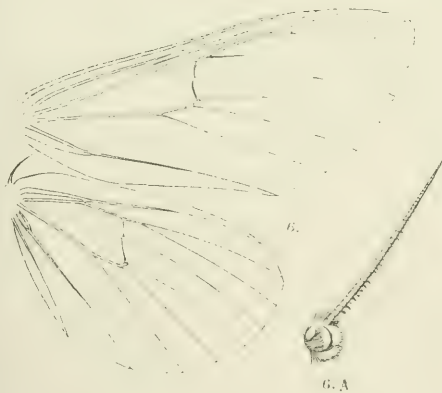
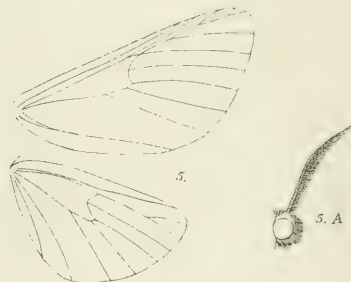


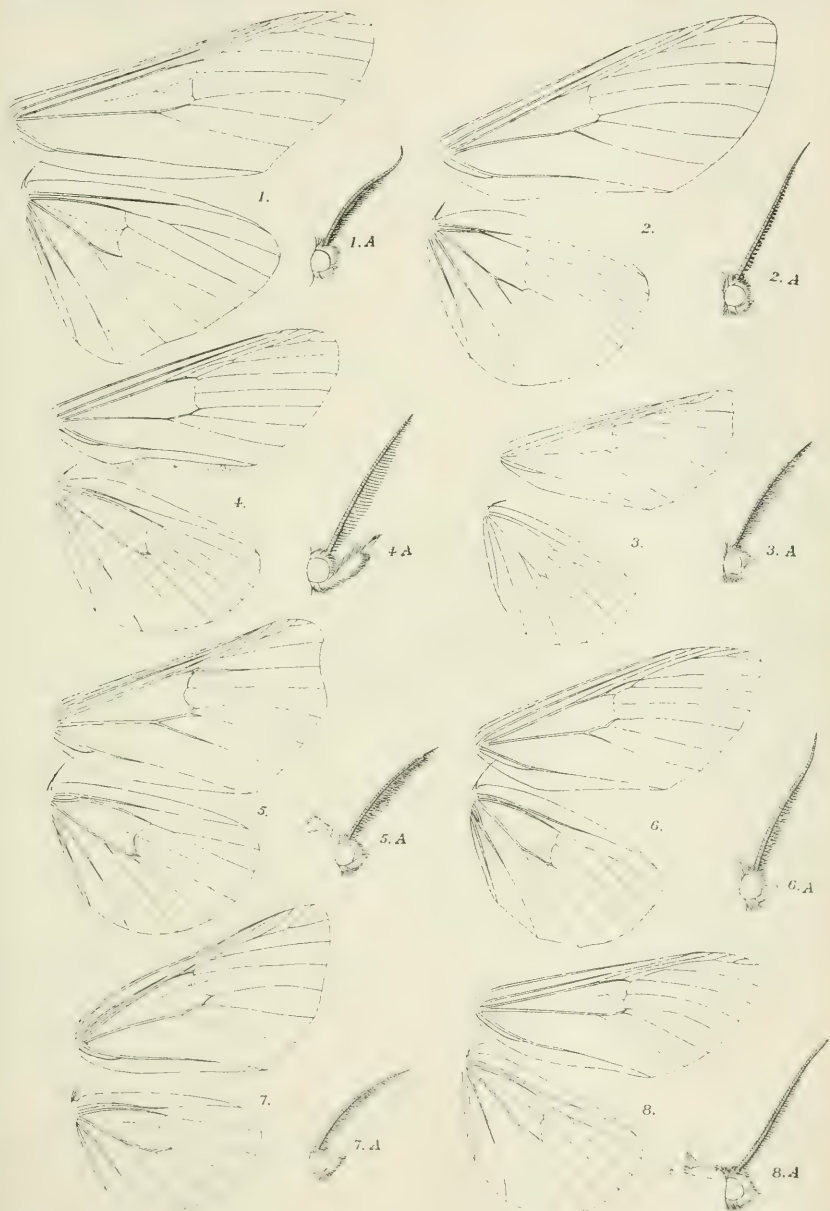


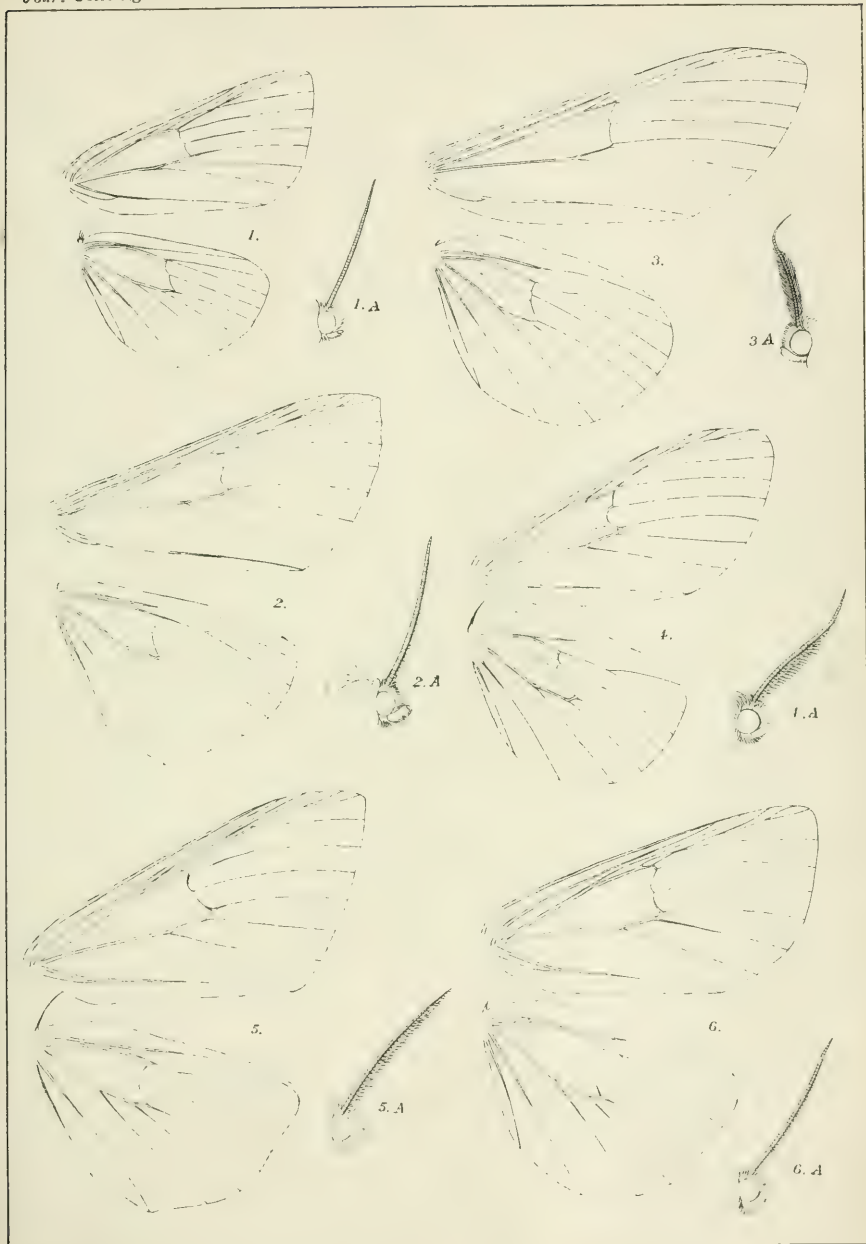




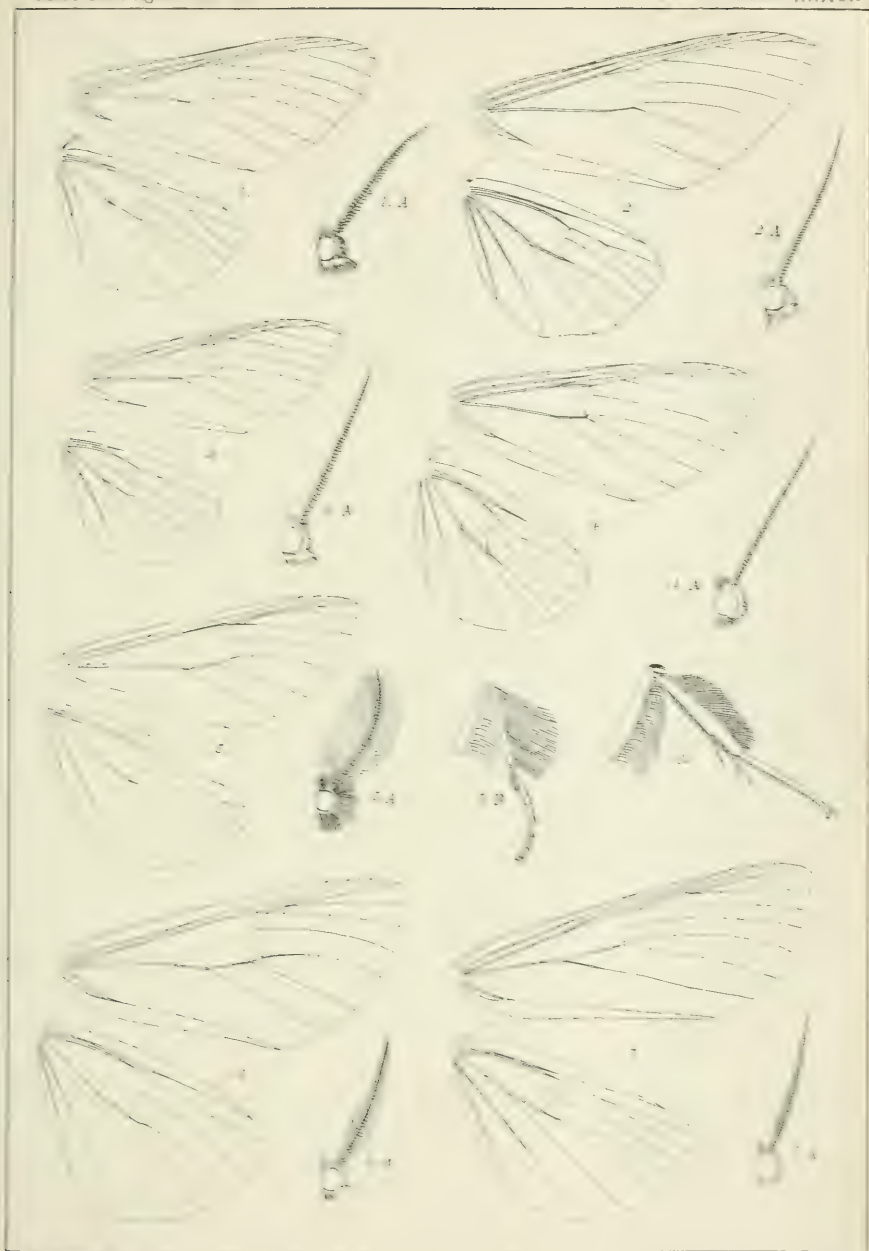












**Studies of the Structure and the Nuclear Division
in a Japanese species of *Opalina*,
O. japonica, nov. sp.**

By

Takesi Sugiyama.

(From the Laboratory for Agricultural Zoology,
Director: Professor CHIYOMASTU ISHIKAWA.)

With Plates XL-XLII and one Text-Figure.

In the year 1915, Professor CHIYOMASTU ISHIKAWA suggested that I should study the Japanese species of *Opalina*, with the remark that it would be very interesting to compare it carefully with those of the European and American species, especially as the former have been so thoroughly worked out by M. METCALF, so that their structure, the nuclear division, and the reproductive processes have, to a great extent, been brought to light. These are the points I have to elucidate, and especially the processes connected with the conjugation and the reproductive cycle, which, in spite of the beautiful work of METCALF, are still to a great extent not cleared up. I regret to say, however, that all my endeavours on these latter points have failed to add anything to the observation of my predecessors. Professor ISHIKAWA not only guided me step by step, but also gave me constant advice during the course of the work and in preparing the manuscript. For all this, and also for permitting me to devote so much time to this study, I have to thank him as well as the Dean of the College, Professor Y. KOZAI, most heartily. To Professor N. YATSU, who gave me advice as to the staining and sectioning, and to Dr. J. MACHIDA, my thanks are also due.

All the work was done in the Zoological Laboratory in the College of Agriculture. The results I obtained were mostly on the structure of the

animal as well as on the nuclear division, all my trials in search of the conjugation having so far failed to give me material enough for the investigation. Since the search for the conjugating individuals in the rectum of tadpoles of the grass-frog caught in ponds, ditches, and in rice-fields, failed me, I injected, during the spring of 1916 and 1917 the cyst taken from the rectum of the adult frog, and found many small individuals hatching out from them, and freely swimming in the rectum of the tadpoles; but the conjugating individuals were so few, that no successful observations could possibly be made. I hope, however, that my injection experiments, when long continued, will give me enough materials for my work, and so that I may be able to report some results in this line of investigation at a future opportunity.

The species on which the following observations were made is a multinucleated one, differing from all the known species in its shape, the relative diameters and in the number of the nuclei, but especially in the mode of division of its body. These differences justify me in distinguishing it from all the others, so far as they are known to me, and to name it the Japanese species of *Opalina*: "*Opalina japonica*."

In addition to the present species, several others were found in our grass-frog, most of which appear to be new. These I hope to describe in a separate paper.

Materials and Methods.

"*Opalina japonica*" can be obtained abundantly from the rectum of the Japanese grass-frog, *Akagaeru* (*Rana japonica*), but is never met with in our edible frog (*Rana esculenta*). It is, however, to be found in the rectum of *Bufo formosus*, a fact which will appear rather curious, as it does not occur in the edible frog which of course is much nearer related to the grassfrog than the toad. This curiosity can possibly be explained by the fact that the oviposition of *Rana japonica* and *Bufo formosus* takes place nearly at the same time, i. e. in early spring, February and March, and in the same place, so that the tadpoles of both *Rana* and *Bufo* are found swimming together in the same stagnant water. This causes the cysts of *Opalina japonica* to be eaten by the tadpoles of both animals, whereas our edible frog lays eggs at

a much later season, i. e. in May and June. This appears to be the reason why the same *Opalinae* are found in both the grass-frog and the toad, but not in the edible frog.

The parasite is found chiefly in the upper part of the rectum scattered between the rectal contents and the rectal wall, but it also occurs in the terminal portion of the intestine, where it is sometimes found abundantly, as shown in the photograph, Fig. 3 Pl. XL.

All the species of *Opalina* which METCALF studied show that the parasites are found only in the upper portion of the rectum, and when these are found in the lower portion of the small intestine it is considered only as an abnormal condition. On this point he says: "In frogs or toads which have been dead for some hours, the *Opalinae* are often found also in the lower part of the intestine, and occasionally, in frogs that were evidently diseased, I have found the posterior part of the intestine to contain some *Opalinae*. Several species of *Opalina* have been reported from the intestines, as well as the recta, of their host. It is possible that these reports are based on observations upon diseased frogs and toads, or upon those that were dead sometime before they were examined." As now stated I can neither accept this statement of METCALF, who considers the occurrence of *Opalinae* in the lower part of the intestines as an abnormality, nor the report of LÉGER & DUBOSCQ, in which *Opalina saturnalis* is stated to occur in the whole intestine of *Bombina orientalis*.

The *Opalina* is generally not found alone, but in company with a species of a small Nematode, a Distoma, a *Nyctotherus* and a *Balantidium*. But when one species of *Opalina* is found in a rectum, no second species of the genus occurs in the same.

For the study of the living animal, PÜTTER's solution was used as a culture medium, but the normal salt solution (0. 6%) and LOCKE's fluid were also used. The first of these seemed to be the best, but the animal kept in it did not live longer than four days. Pieces of the wall of the frog's rectum and the rectal contents added to the above solution, proved to produce better results; the parasites living over nine to eleven days.

For fixing the animal, FLEMING's strong solution, ISHIKAWA's formal-alcohol (to nine parts of 35% alcohol plus one part of 40% of formalin),

SCHAUDINN's alcoholic corrosive-sublimate, corrosive-sublimate-acetic acid etc. were used. Among these, corrosive-sublimate-acetic acid gave the best result, so that this fixative was exclusively used for the present work.

Staining the animals *in toto* was also tried. To this end DELAFIELD's haematoxylin, MAYER's paracarmine, GRENACHER's borax-carmine, and Bismarck-brown were used. These, however, gave no clear pictures, so that I gave up the method and always stained them after sectioning.

The sectioning method chiefly used, was to cut the animals together with the rectum. The rectum containing the animals is first cut off from the frog, and when too much contents was found, this was taken out by means of a very fine pointed pincette. It was then fixed, washed well, dehydrated as usual and imbedded in paraffin and cut. By this method, when the rectal contents are carefully taken out, the section can be cut in thickness of 3-4 μ .

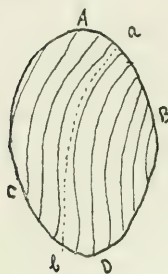
For staining the sections almost all known kinds of dyes were used; such as DELAFIELD's haematoxylin, HEIDENHAIN's iron-haematoxylin, GRENACHER's borax-carmine, iodine-green, aceto-carmine, BIONDI-HEIDENHAIN's three colour mixture, rosanilin followed by iodine-green, safranin O, safranin O followed by acid-violet, acid-violet, HEIDENHAIN's iron-haematoxylin followed by acid-fuchsin, Bismarck-brown followed by acid-violet, methyl-green S-fuchsin, and nigrosin. The best differential staining was obtained with BIONDI-HEIDENHAIN's three colour mixture, while the finest details of structure were seen with HEIDENHAIN's iron-haematoxylin followed by acid-fuchsin and Methylgreen S-fuchsin.

For the study of the sections, the light from a Graetzinlight gas lamp was used as an illuminant, and sometimes NERNST's electric lamp (0.5 Amp) was also used. In both cases, a green screen glass was put at the diaphragm of the microscope, which intensifies the brightness of the image.

I. Morphological Observations.

The body strongly flattened, but not so flat as *O. ranarum*, showing a nearly oval shape in side view. In Text-fig. 1 the morphological anterior end is represented by *A* to *B* and the posterior end by *C* to *D*. The rows of basal granules of cilia lie longitudinal to the axis (*a-b* in text) of the

body, and the cross section of the body always shows an elongated spindle shape.



Text-fig. 1.

According to BEZZENBERGER's table, *O. japonica* ought to come closer to *O. obtrigona* rather than *O. lata* or *O. ranarum*, as it is longest at a point a little anterior to the middle portion of its breadth. But concerning the proportion between the length and breadth of the body, there is comparatively a large difference between *O. japonica* and *O. obtrigona*, from which latter the former can easily be distinguished by its special remarkable shape in facial views. According to the proportion of the length to the breadth of the

body, it is, however, more difficult to distinguish *O. japonica* from *O. lata* or *O. ranarum* than from *O. obtrigona*. But the measurements given in the table will show that *O. japonica* is sufficiently distinct from either *lata* or *ranarum*.

Species	<i>O. japonica</i>		<i>O. lata</i>		<i>O. ranarum</i>	
Length and breadth of the body	length	breadth	length	breadth	length	breadth
	0.2302 mm.	0.180 mm.	0.300 mm.	0.199 mm.	0.800 mm.	0.600 mm.
Diameter of the nucleus	0.005...0.0062 mm.		0.0049 mm.		0.008...0.010 mm.	

The length and the breadth of 100 specimens of *Opalina japonica* were measured as shown in the table.

Table I.

No.	length	breadth	No.	length	breadth	No.	length	breadth	No.	length	breadth	No.	length	breadth	No.	length	breadth
1	0.23	0.18	18	0.18	0.13	35	0.24	0.15	52	0.22	0.16	69	0.29	0.24	86	0.24	0.16
2	0.22	0.15	19	0.28	0.14	36	0.21	0.16	52	0.22	0.16	70	0.22	0.18	87	0.25	0.22
3	0.22	0.20	20	0.22	0.17	37	0.25	0.21	54	0.22	0.19	71	0.25	0.22	88	0.25	0.22
4	0.23	0.20	21	0.25	0.18	38	0.24	0.22	55	0.24	0.20	72	0.21	0.18	89	0.25	0.16
5	0.23	0.20	22	0.23	0.18	39	0.20	0.16	56	0.20	0.15	73	0.22	0.13	90	0.24	0.16

No.	length	breadth	No.	length	breadth	No.	length	breadth	No.	length	breadth	No.	length	breadth	No.	length	breadth
6	0.20	0.16	23	0.27	0.22	40	0.24	0.22	57	0.20	0.17	74	0.25	0.20	91	0.24	0.15
7	0.20	0.22	24	0.22	0.20	41	0.25	0.18	58	0.24	0.18	75	0.24	0.20	92	0.25	0.18
8	0.22	0.18	25	0.20	0.20	42	0.24	0.18	59	0.25	0.20	76	0.27	0.24	93	0.24	0.16
9	0.18	0.12	26	0.20	0.18	43	0.24	0.17	60	0.22	0.14	77	0.27	0.22	94	0.24	0.16
10	0.22	0.16	27	0.23	0.18	44	0.22	0.15	61	0.18	0.15	78	0.25	0.16	95	0.24	0.18
11	0.20	0.14	28	0.22	0.16	45	0.22	0.18	62	0.17	0.12	79	0.22	0.18	96	0.24	0.21
12	0.22	0.18	29	0.22	0.15	46	0.24	0.21	63	0.19	0.15	80	0.25	0.19	97	0.25	0.21
13	0.20	0.14	30	0.20	0.16	47	0.19	0.14	64	0.25	0.20	81	0.27	0.25	98	0.25	0.20
14	0.23	0.20	31	0.22	0.18	48	0.22	0.20	65	0.17	0.13	82	0.24	0.18	99	0.25	0.20
15	0.27	0.22	32	0.16	0.15	49	0.22	0.17	66	0.25	0.20	83	0.25	0.20	100	0.25	0.22
16	0.25	0.20	33	0.25	0.19	50	0.20	0.18	67	0.20	0.14	84	0.24	0.15	Total	23.02	18.00
17	0.19	0.16	34	0.18	0.15	51	0.22	0.18	68	0.25	0.21	85	0.27	0.25	Average	0.2302	0.1800

The division of the body is preceded by a change of form from an oval to a nearly spherical shape, in which the transverse axis becomes even longer than the longitudinal, so that the animal now appears as if moving with its side directed forward. The division always takes place longitudinally and cuts the animal into two halves. These halves are not quite equal, inasmuch as one half is usually small and semilunar in shape, while the other half is a little larger and smooth quadrilateral. This is the condition in the majority of cases, though exceptions in the size and form of the component halves occur very frequently, as may be seen in Fig. 25. This figure shows a dividing animal in a little more advanced stage, in which separation of the halves is taking place at the anterior end, which is usually the case, as METCALF observed in the division of *O. intestinalis* in the period of gamete formation.

According to the same author, the division of the body in *Opalina intestinalis* has a quite close relation with its nucleus. His statement on this point is as follows:— "Division of the body in *O. intestinalis* is usually longitudinal. In one series of preparation of individuals which were probably slightly abnormal, only one of the two nuclei in each individual having a nucleolus, I found that the condition of the nucleolus gave a criterion enabling one to estimate the relative frequency of transverse division. In individuals resulting

from a transverse division, the posterior daughter cell, when its nucleus completed its division, showed the nucleolus in the posterior of its two nuclei; the anterior daughter cells, in a corresponding stage, showed the nucleolus in the anterior of its two nuclei. Only young anterior and posterior daughter cells can with certainty be distinguished by their form and general appearance. In the preparations of abnormal individuals the nucleolar relations were, without exception, as described in the case of the young daughter cells, and doubtless held good for the older cells. In the case of longitudinal division of the body each daughter cell, when its nucleus divides, shows the nucleolus in the posterior nucleus. Eight per-cent of the individuals on these slides show the nucleolus in the anterior nucleus. We can therefore estimate that sixteen per-cent of the divisions were transverse." METCALF states further that the division of *O. caudata* is usually longitudinal, but the transverse division is of the same character and about as frequent as in *O. intestinalis*.

The direction of the division in *O. japonica* is usually longitudinal, as COHN (1904) and SCHAUTEDEN (1905) have stated to be the case in *O. ranarum*. It appears, however, that the division of the body of *O. japonica* has no apparent relation to the nuclear division as in *O. dimidiata*, in which the longitudinal division resembles that of the binucleate forms where the division of the body is always preceded by that of the nucleus. On this the following statement is given by METCALF:— "The division of the body begins while the parent nuclei are in a late anaphase of mitosis, and the separation of the daughter cells, in normal vigorous animals, is complete during the latest anaphase, or less often during the early telophase, when the daughter nucleus is dumb-bell shaped "

The posterior end of the body is generally rounded, but sometimes it is produced into a point, or even furrowed in, in which latter case a radial arrangement of the cytoplasm is seen in almost all cases. At this end the refractive grains are generally seen carried by a sticky material which is apparently secreted from this part of the body.

The shape of the nucleus is, roughly speaking, spherical or oval, and often shows an irregular outline, the average diameter being estimated to be 0.00573 mm. Their number in one individual varies from one hundred to one hundred and seventy.

II. Histological Observations.

The Ectosarc.—The cilia are nearly of equal length all over the body, no special tufts being recognizable either at the anterior or at the posterior end (Fig. 6), as in the case of *O. saturnalis* as described by LÉGER & DUBOSCQ (1904).

The network of fibers described by TÖNNIGES (1898) in *O. ranarum* and by METCALF in *O. intestinalis*, *O. caudata*, *O. ranarum*, *O. obtrigona*, *O. dimidiata*, and *O. zelleri*, beneath the pellicula, could not be distinguished in this species. But very delicate fibrils stretching transversely between the rows of basal granules from granule to granule can clearly be distinguished, more so in fresh materials than in coloured preparations (Fig. 29). These transverse fibrils are placed on the outer surface of the basal granules, and apparently not in the pellicula, but on the limiting surface of the subpellicular layer. The existence and position of the transverse fibrils confirm METCALF'S (1909) description, but differ from the statement made by MAIER (1903), according to whom these exist superficially in the pellicula.

Different colours taken by the cilia and the basal granules on staining them with the following dyes are shown in Table II. The dyes used are: DELAFIELD'S haematoxylin, HEIDENHAIN'S iron-haematoxylin, borax-carmin, aceto-carmin, BIONDI-HEIDENHAIN'S 3 colour mix., rosanilin + iodine-green.

Table II.

Dyes	DELAFIELD'S haematoxylin	HEIDENHAIN'S iron- haematoxylin	borax- carmin	aceto- carmin	BIONDI- HEIDENHAIN'S 3 colour mix.	rosanilin + iodine-green
Cilia	pale blue	pale blue	unstained	good red	red	unstained
Basal- granule	blue	blackish blue	very pale red	deep red	deep purple	very pale purple
safranin O	nigrosin	acid- fuchsin	Bismarck- brown+acid violet	eosin (yellowish)	gentian violet	acid- violet
red	pale gray or unstained	good red	reddish purple	red	pale	reddish blue
good red	unstained	good red	deep red	deep red	purple	blue

The pellicula shows no characteristics peculiar to this species, being similar to those already described by different authors in many other species. MAIER (1903) described the pellicula as "die äussere Ektoplasmalage" in *O. ranarum*, where the thickness is measured to be $\frac{3}{4}\mu$.

The longitudinal parallel furrows of the pellicula, running between the rows of basal granules, are clearly distinguished both in tangential and in cross sections. The ridges of these furrows in tangential sections appear as longitudinal fibers (Fig. 29), whereas in cross sections, they are seen to be papillary processes (Figs. 6, 7, 13, 14 and 26), the number of which between the rows of basal granules of cilia can be counted as three to four, so that the number of furrows lying between them is naturally two to three, just as in *O. ranarum*, according to MAIER's description (1903).

The staining differentiation of the pellicula and its subjacent ectoplasm is brought out with many stains and shown in Table III.

Table III.

Dyes	BIONDI- HEIDENHAIN'S 3 colour mixture	DELAFLD'S haematoxylin	gentian violet	acid-violet
Pellicula	grayish red.	grayish purple	pale purple	deep purplish red.
Ectosarc.	almost unstained	almost unstained	unstained	pale purple

The subpellicular layer which was first found by METCALF as such in *O. dimidiata*, is not sufficiently marked in the present species, as it is very thin, but the alveolar layer is very well marked and can clearly be seen (Figs. 6, 13). The alveoles which lie in the subpellicular layer are very small, while large ones are found in the remaining ectoplasm, which is thus to be recognized as the alveolar layer. In all the alveoles, whether large or small, only one ectoplasmic spherule is found, a fact which confirms the observation of METCALF on *Opalina intestinalis*. Very good staining dyes which demonstrate the ectoplasmic spherules of my species are DELAFIELD'S haematoxylin followed by eosin, and HEIDENHAIN'S iron-haematoxylin followed by acid-fuchsin (Säure-fuchsin); by the former dyes the wall of the alveole stains purple while the ectoplasmic spherule colours pink (Fig. 6), and with

the latter dyes, the ectoplasmic spherule stains yellowish red and the plasm purplish red (Fig. 7).

The shape of the ectoplasmic spherule is always spherical or nearly so, and very rarely more or less irregular. The best fixing reagent of the spherule in the present species is corrosive sublimate-acetic acid.

For the finer structure of the ectosarc, nothing can be added to the extremely delicate and careful observations of METCALF, which the author fully confirms. It is to be regretted, however, that it was not possible to try methyl violet with our species for the demonstration of the ectoplasmic spherules, as METCALF did with his specimens.

Among the sections stained by HEIDENHAIN'S iron-haematoxylin, some are found to have taken quite different colours from those usually obtained, i. e. the ectoplasmic spherules very deeply black, and the endosarc spherules faintly coloured. Under what circumstances such different colourations were obtained could not be determined (Figs. 28, 30).

In the living animal the spherules fill up the alveoles, but in the sections, they shrink a little, even with the most careful fixation. My investigation on this point is too incomplete to give any interpretation upon the function of the ectoplasmic spherules.

The Endosarc.—There are very minute granules in the endosarc, all of which stain very clearly with HEIDENHAIN'S iron-haematoxylin (Figs. 26-28), Bismarck-brown (Fig. 13), or borax- and aceto-carmin, and very delicate fibrils of network, at the nodal portions of which masses of granules can be seen. These latter colour positively with any plasmic stains. But for the demonstration of the network and the granules, DELAFIELD'S haematoxylin, HEIDENHAIN'S iron-haematoxylin followed by acid-fuchsin, HEIDENHAIN'S haematoxylin, BIONDI-HEIDENHAIN'S three colour mixture, and AUERBACH'S two colour mixture, are specially to be recommended.

Among the network are found alveoles which contain endosarc spherules, and the space between the network and the endosarc spherules is filled with very minute granules, which may be termed "ground granules" and can be clearly stained with iodine-green (Fig. 12).

The endosarc subjacent to the ectosarc seems to be denser than the inner endosarc. The endosarc at the marginal portion of the body appears

to be of equal density, no special dense plasm being observed at the morphological anterior part, although that of the posterior portion is apparently a little looser. Regarding the density of the endosarc in other species, METCALF (1909) described in *O. intestinalis* as follows:— "The endosarc spherules are numerous in the anterior part of the body, where the endosarc itself is denser." I have also observed a fact parallel with this in an undetermined cylindrical form parasitic in the rectum of *Rana esculenta*, in a total preparation of which, stained by DELAFIELD'S haematoxylin, the anterior denser portion colours much darker than the posterior which always remains faint.

In a living animal, both the endosarc and the ectoplasmic spherules appear as very refractive bodies, but the refractility becomes more pronounced when the animal is treated with weak acetic acid.

The diameter of the spherules is found to vary from 0'00166 to 0'0025 mm, and their capacity for different dyes is shown in the following table:

Table IV.

Dyes	HEIDENHAIN'S iron- haematoxylin	DELAFIELD'S haematoxylin	eosin	safranin O	acid-fuchsin	aceto- carmine
Reaction	bluish black	unstained	good red	unstained	good red	good red
borax- carmine	nigrosin	BIONDI- HEIDENHAIN'S 3 colour mixture	Bismarck brown+ acid-violet	rosanilin + iodine-green	gentian violet	rubin S
unstained	gray	purplish good red	violet	bluish purple	bluish purple	good red

Endosarc spherules are more numerous in the denser marginal portion of the endosarc than in the inner looser portion, a fact which does not coincide with the observation of METCALF in *O. ranarum*, of which he says: "Contrary to TÖNNIGES (1898) I do not find the endosarc spherules much, if any, more numerous near the periphery of the body even in *O. ranarum*." I am rather inclined to accept the observation of TÖNNIGES, rather than that of METCALF on this point, since it accords with the fact occurring in *O. japonica*, although

I have not had an occasion to study *O. ranarum* myself. It appears also that the number of the endosare spherules are in direct proportion to the density of the endosare. From this fact it may possibly be considered that the endosare spherules have some function to vital or rather nutritive phenomena. These are in living animals spherical in shape, but in sections, although the majority remain spherical, some are found to assume discoidal, elongated or irregular forms. METCALF (1909 and 1912) states that these spherules, disc-shaped endosare spherules, or endosare plastids, as he calls them, are arranged in regular direction in *O. intestinalis* and *O. mitotica*.

In order to study the finer structure of the spherules, HEIDENHAIN's iron-haematoxylin, BIONDI-HEIDENHAIN's three colour mixture, Bismarck-brown, and some other dyes were used as staining reagents, and the following results were obtained. With HEIDENHAIN's iron-haematoxylin, they take an uniform deep dark, bluish colour, in which by a suitable destaining, the central portion becomes so decolourized that the spherules appear like alveoli (Fig. 31). This also brings out very fine granules in the peripheral coloured portion (Fig. 31), which escaped the observations of MAIER (1903), LÉGER & DUBOSQ (1904), all these investigators describing them as homogeneous bodies; TÖNNIGES (1898)* has, however, found that the spherules in *O. ranarum* are not homogeneous, but have a denser marginal portion, thus making them appear as bubbles, the inner portion of which is according to METCALF (1909) filled with alveolar structure. The statement made by this author is as follows:—"I can confirm his (TÖNNIGES') statements that they (endosare spherules) are discoid shaped, elongated or irregular in form and are of various sizes; that they lie usually (in *O. intestinalis*) in a regular direction in the whole body, the flat side of the disc (when they are disc-shaped) being parallel to the surface of the body, so that in sections parallel to the flattened surface of the body one sees them almost circular, while in other sections they appear almost rod-shaped: that they appear homogeneous when strongly stained with (most) anilin dyes; but not with (well extracted) iron-haematoxylin; that they show an alveolar (?) structure"

In well made preparations we can generally observe very faintly coloured

* The original description of TÖNNIGES was not accessible to me, so that I am obliged to make this statement according to METCALF.

grains in the clear central portion, but no alveolar structures like those observed by METCALF (1909) in *O. ranarum* were recognized.

The division of the endosare spherules is described by several authors; that of *O. ranarum* by TÖNNIGES (1898), that of *O. dimidiata* by KUNSTLER & GINESTE (1905). This is, however, denied by METCALF (1909), who came to the following conclusion:—"At the time I wrote my preliminary paper (METCALF, 1907) I assumed that the frequent dumb-bell shape indicated division, but I now think that these bodies do not divide any more than do the ectosare spherules. One never finds two of either sort of spherule in one or any other indication of division in them." I can only confirm this observation of METCALF, and am inclined to believe that the dumb-bell shape assumed by the spherules is only due to an abnormal condition, and has nothing to do with the division, since I have never been able to see any spherules of this shape in fresh materials, which always appear to be spherical.

Just as with the ectoplasmic spherules, the function of the endosare spherules was not determined.

The Excretory Organs.—The excretory organ could not be recognized. All the methods tried to demonstrate the existence of it were in vain, both in adult and young animals. METCALF (1907) who first made known the existence of the organ in three species of *Opalina* (*O. obtrigona*, *O. caudata*, and *O. intestinalis*), is inclined to believe that the organ is well developed in all the cylindrical species, while it is reduced or even absent in flattened forms.* Thus while the organ is found in *O. intestinalis*, *O. caudata*, and *O. dimidiata*, it is rudimentary in *O. obtrigona*. The absence of the organ in *O. japonica* is perhaps to be looked upon as an additional fact which supports METCALF's view.

III. Cytological Observations.

The Resting Nucleus.—The number of nuclei in one individual was counted to be about from one hundred to one hundred and seventy as previously described, and the diameters of the nuclei are from 0.0050 to 0.0062 mm. In general, the structure and the kinetic phenomena more or

* METCALF, 1912, p. 82: "Probably all cylindrical species have well developed vacuoles. In the flattened species vacuoles are reduced or absent."

less resemble those of *O. intermedia*, studied by METCALF (1914). The nuclei lie in the endosare, and their nuclear membranes are firm and strong, showing no structure, and never disappear during the division.

There is a true resting stage of the nucleus entirely free from kinetic activity. In this the number of chromatin masses which lie just beneath the nuclear membrane is from two to several; they are all trophic in function and can be called "trophochromatin" or cyano-vegetative chromatin (HERTWIG, 1907, and METCALF, 1914), while an alveolar or rather a reticular structure seen within the nucleus is probably the linin which fills up the entire nuclear body, on the fibers and nodal portions of which, grains or masses of chromatin are suspended. These latter vary very much in size, and can be considered as the idio-, erythro- or generative chromatin, which are destined to form the chromosomes.

In *O. macronucleata* and *O. lata*, BEZZENBERGER* observed masses of vegetative chromatin which, however, were not demonstrated by him as vegetative. In *O. ranarum*, NERESHEIMER described the nucleus in agamogeneous generation as follows:—"Während des ganzen vegetativen Lebens findet man die *Opalina* im Froschrectum immer annähernd gleich aussehend, wie sie ZELLER und TÖNNIGES beschrieben haben. An gefärbten Tieren fällt der geringe Chromatingehalt der Kerne sofort auf. Ein wabiges achromatisches Gerüst der Kerne ist stets gut zu erkennen, dem wenige minimale Chromatinpartikelchen eingelagert sind." The nucleus in encysting individuals described by him in the same paper, shows a similar structure to that of my species.

If the sections are stained with BIONDI-HEIDENHAIN's three colour mixture or methylgreen S-fuchsin, the peripheral masses of chromatin (vegetative), nuclear membrane, and linin fibers colour purplish red, while the inner generative chromatin grains take a fresh green colour (Figs. 14-21), and when these are stained with Heidenhain's iron-haematoxylin followed by acid-fuchsin, both the vegetative and the generative chromatins stain bluish black, while the nuclear membrane and the linin become dark purplish red (Fig. 11). KAZANZEIT (1910) obtained the colour differentiation between macro- and

* BEZZENBERGER, 1904, P. 163: "Das Chromatin ist in 2-3 grösseren Plaques der Oberfläche eingelagert, der übrige Kerninhalt zeigt feinen wabigen Bau."

micronucleus in *Loxodes rostrum*, in which the macronucleus takes a red colour, while the micronucleus stains green. No such differentiation was observed in my species.

METCALF observed the peripheral masses of vegetative chromatin in *O. obtrigona* and writes in his paper (1909), in the explanation of the plate, as follows:— "The network with thickened nodes is superficial and is probably chromatin, but the achromatic foam, filling the whole nucleus, presents much the same appearance. Each nucleus shows from two to six discoid masses of chromatin upon the nuclear membrane." Here the "two to six discoid masses of chromatin" would be the vegetative chromatin, and "the network with thickened nodes" may be compared with the generative chromatin of my species. In a later paper (1914), in which he speaks of the same condition in two, binucleate species (*O. intestinalis* and *O. caudata*), the following description is given:— "The phenomena observed in *O. intestinalis* and *O. caudata* suggest that HERTWIG's distinction between tropho-chromatin and idio-chromatin may here apply. The massive chromatin which is thrown away bodily before the sexual phenomena are completed, seem plainly to be not reproductive. Accepting HERTWIG's term we will call it trophic."

PFITZNER (1886) considered the nuclei of *Opalina* as meganuclei, and not micronuclei, and NERESHEIMER (1907) observed the trophochromatin in *O. ranarum* but did not consider it as such. According to his description, the generative nucleus develops from the extruded chromatin (generative chromidia). The extruded chromatin is, however, as stated above, considered by METCALF (1909) to be vegetative chromatin, an assumption with which I fully agree.

It may be stated that the nucleus of *Opalina* is still in an undifferentiated primitive condition, in which the generative and the vegetative chromatins are still contained in one and the same nucleus, the peripheral masses of chromatin representing the vegetative and the inner masses the generative. These two kinds of chromatin masses may be supposed to have become separated into two independent nuclei, forming the macro- and the micronuclei of higher Ciliata.

The Centrosome, the Spindle, and the Mechanism of Mitosis.

The center of the kinetic activity seems to belong to that category of

unicellular organisms of which CALKINS (1903) describes it as intra-nuclear, though, neither centrosome nor centriole are to be observed in this species, either inside or outside of the nuclear membrane. The spindle, consequently, is composed of linin, as in the case of *Didinium nasutum*, according to PRANDTL's* description, and originally differs from those found in *Noctiluca* and Metazoon cells, in which the spindle fibers originate from archoplasm or central plasm. METCALF states in *O. intestinalis*, that the spindle is composed of two kinds of material, and says:— "The mitotic spindle in *Opalina* is also interesting in the fact that it is formed from both chromatic and achromatic material. In the resting nucleus the achromatic foam fills the whole nucleus, a network of chromatin fibrils being also present over the surface of the nucleus just beneath the nuclear membrane. The appearance of longitudinal striation in the dividing nucleus is due to the emphasizing of the longitudinal strands of the chromatin net and the longitudinal films of the achromatic foam. The spindle, therefore, is composed of a central achromatic portion and a superficial chromatic portion." No such chromatic portion can be recognized in the spindle of my species.

In the case of division, the spindle fibers and the nuclear membrane seem to take an active part, the chromatic portion a passive one; especially in the stage of anaphase or telophase, it is easy to imagine that the former structures elongate intensively at the median portion of the dividing nucleus, forming the connecting strand, and push the chromatic portion to the poles of the nucleus.

According to METCALF (1909), all the parts of the nucleus, however, seem to be active, especially the nuclear membrane and the chromatic spindle fibers. In our species, although the nuclear membrane appears to participate in this activity, the chromatic spindle fibers can not play any part in it, as no such structures can here be recognized.

The Nucleolus.—A nucleolus was observed by METCALF (1909) in *O. intestinalis*. In the present species no structure can be detected which could possibly be counted for as such, all the methods tried to demonstrate it having so far failed.

*PRANDTL (1906): "Die Spindelfasern sind somit aus dem Kernretikulum gebildet."

The DIVISION of the NUCLEUS.

Prophases.—When the nucleus enters into the prophases, the generative chromatin begins to gather at the nodal points of the linin network, increasing the density and the staining capacity, and forming masses of the chromatin which unite very irregularly with each other, and show a special appearance; this stage can possibly be compared with the coil stage of Metazoon cells. Similar stages were observed by NERESHEIMER (1907) in *O. ranarium* and by BEZZENBERGER (1904) in *O. macronucleata*. In Fig. 37, the chromatin grains are seen gathered at the nodal points of the linin network, becoming larger and thicker, and finally uniting with each other as shown in Figs. 37 and 38. Meanwhile the nucleus becomes elongated, as in Figs. 39 and 41 or 42, and the linin fibers now begin to be placed parallel to the longitudinal axis of it. In this process it appears that those fibers which are placed transversely become fainter and disappear, while the longitudinal ones remain. In *O. intestinalis*, a similar process is stated by METCALF to be produced by the chromatin network, and not by linin fibers as in my species.

It sometimes occurs however, though very rarely, that some of the transverse linin fibers persist till the middle prophase stage where these are still to be recognized (Fig. 16). At any rate, the linin fibers lose the reticular condition and turn to form the longitudinal fibers of the spindle. Both extremities of these fibers seem either to touch the nuclear membrane or come close to it, the space between them and the latter being occupied by masses of generative and sometimes also by vegetative chromatin (Figs. 16, 17, 41, 42, and 43). The masses of vegetative chromatin sometimes break up into smaller irregular ones as in Fig. 38, or sometimes, on the contrary, unite to form two to three large masses as illustrated by Fig. 40, these facts make it probable that the vegetative chromatin plays no part in the process of mitosis. In spring, at a stage of the encystment or a little before it, one can easily recognize a portion of vegetative chromatin lying outside of the nuclear membrane (Figs. 33, 34), which can be looked upon as the result of extrusion out of the nucleus, and although the process of extrusion was not directly observed in my case, it can certainly be conjectured that the compact spheres of vegetative chromatin seen inside or outside of the nuclear membrane at this stage represent the stages of such an extrusion as those observed by

many other authors, especially by METCALF. What makes it more probable, is the fact that in sections of animals obtained in summer or autumn, one can never observe anything like it. The extruded chromatin persists (in cytoplasm) for a while after the extrusion, but later on, it diminishes in density and staining capacity, and at last degenerates. NERESHEIMER (1907), as previously mentioned, assumed the extruded chromatin in *O. ranvrum* to be a generative and destined to form the reproductive nucleus. His statement on this point is as follows:— “Der übriggebliebene Teil der Chromidien (viz. the extruded vegetative chromatin in *O. japonica*)*, den wir nunmehr als Sporetien oder Gametochromidien anzusprechen haben, verteilt sich zunächst in Gestalt kleiner rundlicher Körnchen durch das ganze Plasma. Unterdessen ist die fortwährende Quer- und Schrägteilung der *Opalinen* ohne Unterbrechung weiter gegangen und hat zur Bildung schon wesentlich kleinerer Individuen mit weniger Kernen geführt. “...” Das Sporetium, das sich vorher in Form kleiner Teilchen frei im Plasma befunden hatte, findet sich nun zum grössten Teil in diese Alveolen eingelagert; meist sieht man einen grösseren Chromatinbrocken, manchmal auch mehrere in einer Alveole. “.....” Bei *Opalina* verteilt sich zunächst das Chromatin in Form feiner Körnchen regelmässig durch die ganze, immer kompakter werdende Plasmakugel: man sieht eine Kernmembran auftauchen und ein Kerngerüst sich bilden.” These statements of NERESHEIMER are not in accordance either with the observations of METCALF (1909) or with my own. The chromatin masses which are extruded from the nucleus into the cytoplasm before the sexual reproduction sets in, degenerate sooner or later. This fact makes it probable that these chromatin masses are vegetative and not generative, and are comparable to the macronuclei of the higher Ciliata, which as is known, also degenerate before the conjugation of the micronuclei begins.

METCALF (1909) described the manner of extrusion of the vegetative chromatin as well as its degeneration in *O. intestinalis* as follows:— “In living nuclei at this stage (encystment stage), which are getting rid of their chromatin in this peculiar manner, one observes two large balls or discs which by staining are clearly shown to be composed of chromatin. Occasionally

* The parenthesis is mine.

instead of two such chromatin spheres one finds three, one or two of these being smaller. In other cases but one sphere is found, but in these cases another may have been present and have been extruded. The rest of the contents of the nucleus lie in the form of granules, generally in an hour-glass-shaped group, transversely between the two chromatin spheres when two are present. "....." These compact spheres of chromatin are extruded from the nucleus into the cytoplasm and there degenerate." The compact spheres of the vegetative chromatin in my species are not always two to three in number as he states, but are found sometimes in larger numbers, a fact which makes it probable that all of the vegetative chromatin elements are extruded from the nucleus, leaving only the generative, before the copulation sets in. Further observations on this point are, however, required to ascertain the validity of such an assumption.

The generative chromatin which, as above stated, forms the so-called spirem now begins to break up into fragments, of rather irregular shape, but showing a more or less elongate form. These may perhaps be compared to the "chromosomes," and will be called so, but only for the sake of convenience, since they are quite irregular both in size and shape (Fig. 40). These chromosomes then migrate towards the equator of the nucleus, and form a structure which may be compared to the equatorial plate of Metazoon cells (Figs. 41, 42).

The equatorial chromatin ring described by LÉGER & DUBOSCQ (1904) in *O. saturnalis*, was not observed in this species. The equatorial plate of *O. intestinalis* is according to METCALF (1909) very irregular and ill-defined as compared with mine.

Anaphases.—The longitudinal splitting of the chromosomes was not observed, but the transverse division of the same were occasionally seen to occur. As to the splitting of the chromosomes, METCALF writes:— "At present (1909) we can only say that splitting of the chromosomes does not occur at the equatorial plate stage, that it may occur in the telophases, and that in the anaphases the daughter chromosomes seem to be paired as in Metazoon mitosis."

The longitudinal fibers now commence to move toward the poles, thus the division of, or simply the separation of the chromosomes, takes place

bodily. In the case where the transverse division of the chromosomes occurs (Figs. 42, 43), the daughter chromosomes are very irregular, both in their movement and size (Figs. 42, 44, 45, and 46).

In *O. saturnalis*¹ and *O. antillensis*², two sets of chromosomes are described,—the massive and the granular chromosomes, both of which lie just beneath the nuclear membrane, which may be compared with the vegetative and generative chromatin of my species. But my vegetative chromatin does not form, as already described, chromosomes during the mitosis, but is carried bodily into the daughter nuclei and is thus not to be compared with the massive chromosomes of these authors. Similar facts were also observed by METCALF (1914) in the nuclei of one of the undetermined multinucleate species of *Opalina* (which he calls *O. intermedia*) infecting the *Bufo helophilus* of California; he states that the vegetative chromatin of this species does not form chromosomes at all. He also states the absence of the massive chromosomes in another multinucleate species.

It is not possible to count the number of the chromosomes, as the form and size of the same are very irregular, the calculation made in a hundred individuals gave me no definite result.

During the elongation of the nucleus the chromosomes begin to migrate towards the poles. This takes place, however, very irregularly, as shown in Fig. 43, where the peripheral vegetative chromatin is seen to migrate bodily to the poles. At the same time, the chromosomes too are seen to migrate towards the poles, but their movement is so irregular that while some of them have already reached the poles, others are still at the equator or near it (Figs. 44, 45, 48, and 49).

At the end of the migration, when all the chromosomes come to be placed at the poles, very fine thread-like fibers are seen to connect the chromosomes of the opposite poles in pairs (Figs. 50-52). These fibers presumably correspond to the connecting fibers ("Verbindungsfasern" of German authors) of the Metazoon cells, and may be so termed. The connecting fibers seem to originate from the linin-substance of the nucleus as described by ISHIKAWA

1. LÉGER & DUBOSCQ: Notes sur les infusoires endoparasites, III. *O. saturnalis*, Archiv. Zool. T. 2.

2. M. METCALF: Notes upon *Opalina*. Zoolog. Anzeig. Bd. XLIV. 1914.

(1897, p. 306) in the nuclear division of *Noctiluca miliaris*. Similar fibers were observed in other species of *Opalina*, as for instance in *O. intestinalis*, these are described by Metcalf (1909) as chromatin fibers; and in *O. antillensis*, the same fibers are described by the same author as "easily staining threads" (METCALF, 1914, P. 535). In *Didinium nasutum*, PRANDTL¹ observed the connecting fibers (Verbindungsfäden or Verbindungsfasern) and states as follows:— "Die zahlreichen Verbindungsfasern erscheinen anfangs gekrümmt und locker, allmählich beim weiteren Auseinanderrücken der Tochterkerne wachsen sie zu einem dichten, starren, mit Eisenhämatoxylin tiefschwarz gefärbten Strang aus, dessen Fasern wohl durch Verkleben mehrerer Spindelfasern zustande gekommen sind." Moreover, the spindle fibers in *Didinium*² are composed according to PRANDTL, of nuclear reticulum. It will be remarked that the "Verbindungsfasern" which originate by the "Verkleben mehrerer Spindelfasern" are to be compared with those of *Opalina*, but differ from those of *Noctiluca* where these originate from the linin substance.

The nucleus now becomes spindle shaped, with both the poles strongly pointed (Figs. 50–53), and the chromosomes come to be placed in parallel position (Fig. 53). But as the stage advances, the shape of the nucleus changes from a spindle to an elongated cocoon, the long diameter of which now measures more than that of the spindle-shaped.

Telophases.— During this stage the elongation of the nucleus continues, this chiefly takes place at the median portion which continuously elongates, becoming gradually narrow, while the terminal ends get larger (Figs. 55–57). In Fig. 55, we see just the commencement of the elongation at the median portion, a slight incurving being observable on both sides of the figure. These are more pronounced in Fig. 56, and gradually increase successively in Figs. 57 to 61, at the same time the gradual bulging of the terminal portion of the nuclei is to be observed. It is to be noted that in this not only the median portion of the nucleus elongates, but also the mass of the plasm moves towards both ends.

¹ H. PRANDTL: Die Konjugation von *Didinium nasutum* O. F. M. Archiv f. Protistenk. Bd. VII, 1906, p. 234.

² PRANDTL: 1906, p. 234: "Die Spindelfasern werden somit aus dem Kernretikulum gebildet."

Not only does the median portion of the spindle elongate, but it also bends, as is shown in Fig. 59 or 60. Similar phenomena were observed by many previous authors in different animals, so by PRANDTL (1906) in the micronucleus of *Didinium nasutum*, by R. HERTWIG and G. CALKINS in that of *Paramecium*, and by C. ISHIKAWA in *Noctiluca* (ISHIKAWA's Figs. 15, 16). PRANDTL says: "In frühen Perioden der Metaphasen gewinnt die Spindel manchmal durch Krümmung ihrer Achse ein etwas sichelförmiges Aussehen." The long axis of the spindle not only bends but sometimes coils up and forms a loop, this condition was also observed by METCALF in *O. intestinalis* where the connecting strand remained till the beginning of the next mitosis. In our species, although the connecting strand does not remain, its torn ends can be recognized at the corresponding ends of the nuclei, which enables us to distinguish premitotic nuclei from the postmitotic (Figs. 70, 71.).

The connecting strand does not only coil up, but the median portion of it often bulges out (Figs. 62 and 63), a fact which was also observed in other species of *Opalina*, as well as in various other Protozoa, as for instance by HERTWIG (1896) and by CALKINS & CULL (1907) in the division of the micronucleus of *Paramecium*, and by C. ISHIKAWA (1894 and 1899) in the nuclear division of *Noctiluca*, where ISHIKAWA says:—"When the division has pronounced, the median portion of the archoplasmic spindle is swollen up a little. This part of the spindle is left behind after the complete separation of the nuclei in the form of a small diagonal figure." Although the swelling generally contains no chromatic elements, some are observed with such (Fig. 63), and these are always proved to be vegetative by their behavior.

As is suggested to me by Prof. ISHIKAWA, the elongation of the nucleus is positively carried on by the active extension of the connecting strand, and the bending or the formation of the coil produced by the same, as well as the bulging out of its median portion, is most probably caused by the nature of the cytoplasm adjacent to the nucleus, the cytoplasm at both the ends of the dividing nucleus acting as resistance to the movement of the daughter nuclei. The relative distance of the nuclei soon after the division, as compared with the end portion of those during the same, can also be ascribed to the same cause (compare Figs. 59, 60 with Fig. 71).

At the end of the division of the nucleus, when all the chromosomes

enter into the daughter nuclei, the parallel arrangement of the same can still be recognized (Figs. 60—68). No transverse constriction of the chromosomes was observed previous to the division. In most cases the condition of the chromosomes at the two poles are similar; only in very few instances the chromosomes in one pole are seen to break up into many small bodies, while those in the other still keep their parallel arrangement (Fig. 63).

When all the chromosomes have finished their migration, the achromatic fibers (connecting fibers also) turn to form the linin network of the nuclei of the following stages, the nuclear membrane, as already stated, being very stout and never disappearing during all phases, as is usual in all other Protozoa.

Resting stage.—The chromosomes now begin to lose their density gradually, and the constituent granules come again into view. These occur sometimes in comparatively earlier stages, as shown in Figs. 59 and 60, but generally in late telophases (Figs. 62, 63, and 69). In a few cases, however, the chromosomes keep their parallel arrangement till the last stage (Fig. 66). In *O. intestinalis* and *O. caudata* METCALF (1909) describes a stage which is comparable with the dispireme of Metazoon nuclei, but in our species such a stage was not observed, the linin fibers, soon after the division of the nuclei, assuming the reticular structure with the chromatin granules distributed over them, and thus the nucleus enters into the resting condition, without passing through the dispireme stage (Figs. 67—71).

Vegetative chromatin in the postmitotic nucleus shows the same character as that of the premitotic nucleus, lying on the inner surface of the nuclear membrane in the form of several masses of discoidal bodies as above mentioned (Figs. 70, 71).

Summary.

1. *Opalina japonica* ought to come close to *O. obtrigona*, according to BEZZENBERGER's table, but concerning the proportion of the length and the breadth of its body, the diameter of the nucleus, and especially the mode of the division of its body, it is more closely related to *O. ranarum* and *O. lata*, from both of which it is, however, distinguished by the characters above described, so that it fairly forms a new species.

2. *O. japonica* can be found in the recta of both *Rana japonica* and *Bufo formosus*, as the oviposition of both animals takes place in the same season and in the same place, which makes the tadpoles live in the same place, thus giving them the opportunity of swallowing the cysts discharged from the adult frog or toad. The reason that it is not found in *Rana esculenta* is perhaps to be sought in the different habits of this frog from that of the above two.

3. The division of the body always takes place longitudinally, the separation of the halves beginning at the anterior end.

4. The pellicula is distinct and homogeneous and has longitudinal furrows on its surface, the number of which between two rows of the basal granules is two to three as stated by MAIER in *O. renarum*, but no transverse striation can be observed in it, thus making the muscular nature of the longitudinal ridges of pellicula, as maintained by ZELLER, unacceptable.

5. The network of fibers as described by TÖNNIGES and METCALF in the subpellicular layer was not observed, but delicate transverse fibrils can clearly be distinguished at the peripheral portion, stretching between the basal granules.

6. The alveolar layer is marked in this species, and the alveoles whether large or small, always contain only one ectoplasmic spherule, as observed by METCALF in *O. intestinalis*.

7. The endosarc subjacent to the ectosarc is denser than the inner endosarc. The endosarc spherules lie more numerous in the peripheral denser portion than in the inner looser portion, a fact which coincides with METCALF's description in *O. intestinalis*. This possibly shows the relation existing between the endosarc spherules and the nutritive function of the animal.

8. The endosarc spherules are not homogeneous as described by MAIER or LÉGER & DUBOSQ, but are alveolar as TÖNNIGES and METCALF observed. Division of these as assumed by TÖNNIGES and KUNSTLER & GINESTÉ can not be accepted, the dumb-bell shapes taken by the spherules are to be considered rather as an abnormal condition.

9. The chromidium could not be distinguished in the endosarc; the structure described as such by NERESHEIMER is probably the endosarc spherules. The extrusion of the chromatin into the cytoplasm takes place as NERESHEIMER

describes; but the extruded chromatin is apparently a vegetative one and does not persist in the cytoplasm as a generative chromidium, but degenerates sooner or later. This confirms the description of METCALF rather than that of NERESHEIMER.

10. The centrosome and the nucleolus were not recognized.

11. An excretory organ was also not seen. Refractive coccus-like grains attached at the posterior end of the body by a sticky material can possibly be taken as a phenomena produced by the excretory function.

12. Two kinds of chromatin are distinguishable in the nucleus, a vegetative and a generative, the former, in the resting stage, lying on the inner surface of the nuclear membrane in the shape of two to several discoidal masses, while the latter exists as grains or small masses scattered over the linin fiber, and destined to form the chromosomes.

13. The vegetative chromatin of *Opalina* may be compared with the chromatin of the macronucleus of higher Ciliata, and the generative chromatin with that of the micronucleus. Both chromatins are found in one and the same nucleus, but can be distinguished by a proper staining method.

14. Thus a nucleus of an *Opalina* can be considered as in an undifferentiated primitive condition.

15. During the division the vegetative chromatins are carried bodily to the poles, the generative ones form very irregular chromosomes, the shapes and sizes of which are very irregular, which makes a calculation of their number utterly impossible.

16. Longitudinal splitting of the chromosomes can not be observed, but the transverse division of the same seems to occur. The equatorial plate is very incomplete, just as in *O. intestinalis*.

17. Connecting fibers originate from the linin-substance, these are not so long as those of *O. intestinalis* and do not make a coil as in that species.

18. There is no defined dispireme stage between the telophase and the resting stages.

19. The nuclear membrane is very stout and strong, and does not disappear during the mitotic cycle as is usual with all other Protozoa.

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EXPLANATION OF PLATES.

Plate XL.

All the figures are photographic reproductions made by the author.

Fig. 1. Transverse section of a frog's rectum, which contains very many individuals of *O. japonica* together with some other kinds of parasitic animals. In the Figure, most of the *Opalinae* appear as spindle shaped bodies. Magnified 27 times.

Fig. 2. Several adult *Opalinae*, showing the general shape of the body, taken from freshly killed material; LEITZ's dark-ground illuminator. Magnified 70 times.

Fig. 3. Transverse section of the terminal portion of a small intestine of a frog, showing many *Opalinae* contained in it. Magnified 28 times.

Fig. 4. Part of a transverse section, given in Fig. 1, with a higher magnification. Most *Opalinae* appear as spindle shaped bodies, in which the nuclei are clearly to be seen. Magnified 225 times.

Fig. 5. Part of a cross section of *Opalina*, showing the endoplasmic structure. Magnified 1300 times.

Plate XLI.

All the figures are drawn with the aid of ZEISS' drawing apparatus. Figs. 6-12, and Figs. 15-21, with ZEISS' apoch. 1.5 mm objective and compensating ocular 8; Figs. 13 and 14 with ZEISS' 1/12 objective and ocular 4.

Fig. 6. Part of the transverse section of *O. japonica*, stained with DELAFIELD's haematoxylin followed by eosin. The endosarc, basal granules, and the chromatin are purple, while both of the ecto- and endosarc spherules, pellicula, and cilia are pinkish red in colour.

Fig. 7. Ditto: stained with HEIDENHAIN's iron-haematoxylin followed by acid fuchsin. Cilia, pellicula, basal granules of cilia, endoplasmic network, and peripheral portion of endosarc spherule are purplish red and the ectoplasmic spherules, yellowish red.

Fig. 8. Ectoplasmic spherules, from the section stained with DELAFIELD's haematoxylin followed by eosin.

Fig. 9. Ditto: from a section stained with Bismarck-brown followed by acid violet.

Fig. 10. Endosarc spherules, from a section stained with DELAFIELD's haematoxylin followed by eosin, the peripheral portion of the spherule is coloured purple while the inner portion is red.

Fig. 11. Part of a section of *O. japonica*, stained with HEIDENHAIN's iron haematoxylin followed by acid fuchsin, showing the colour differentiation of nucleus. The vegetative and generative chromatins are coloured purple, while the nuclear membrane and linin are red.

Fig. 12. Ditto: stained with safranin followed by iodine-green. Endoplasmic spherules, ground granules, network fibers, linin, and the nuclear membrane are stained green, while both the vegetative and the generative chromatins are coloured red.

Fig. 13. Part of a section of *O. japonica* cut transversely to the rows of basal granules of cilia and stained with Bismarck-brown followed by acid violet. Cilia, basal granules, pellicula,

ectoplasmic spherules, peripheral portion of the endosarc spherules, and the linin are blue, while the endosarc is lighter brown and the chromatin dark brown.

Fig. 14. Ditto: coloured with BIONDI-HEIDENHAIN'S three colour mixture. Greenish colour was taken only by the generative chromatin, while the other portions (cilia, basal granule, endosarc network, nuclear membrane, peripheral part of endosarc spherule, and the vegetative chromatin) are purplish red.

Fig. 15. Resting nucleus, from a section stained with BIONDI-HEIDENHAIN'S three colour mixture. The peripheral vegetative chromatin, nuclear membrane, and linin are stained purplish red, while the generative chromatin is green.

Figs. 16-21. Dividing nuclei stained with BIONDI-HEIDENHAIN'S three-colour mixture.

Fig. 16. Early anaphase nucleus. The generative chromatin condenses and forms the chromosome, and the vegetative chromatin is divided into several masses, which are connected to each other by linin.

Fig. 17. The linin forms the spindle fibers which extend between the poles, while the chromosomes are seen migrating from the equator.

Fig. 18. Early telophase nucleus, showing the connecting fibers originating from the spindle fibers.

Fig. 19. Advanced telophase nucleus.

Fig. 20. Late telophase nucleus showing the commencement of its bending.

Fig. 21. More advanced and just before the resting stage; two nuclei are connected by a connecting strand.

Plate XLII.

This plate is reduced to about 5/7 of the original size. In the original plate the Figs. 26-30, 35-71 were drawn with ZEISS' apochromatic 1.5 mm objective and ZEISS' compensating ocular 8, Figs. 31-34 with ZEISS' apoch. 1.5 mm objective and ZEISS' compensating ocular 12, Figs. 23-25 with LEITZ' projection apparatus and LEITZ objective 3. Fig. 22 is a diagrammatic representation. All the other figures are drawn from a section stained with HEIDENHAIN'S iron haematoxylin, if not otherwise stated.

Fig. 22. Diagrammatic figure of *O. japonica*, showing typical shape of the body. The spiral lines show the longitudinal direction of the body which coincides with the morphological axis of it.

Figs. 23, 24. Dividing *Opalinae* showing the typical mode of fission, drawn from fresh materials. Magnified about 150 times.

Fig. 25. Dividing *Opalina*, showing the mode of the separation of the halves taken from fresh material. The two component halves are somewhat differently shaped. Magnified about 150 times.

Fig. 26. Part of a transverse section of *O. japonica*, stained with HEIDENHAIN'S iron haematoxylin. Ridges of pellicula are seen as papillary processes; with the basal granules of cilia stained black. The endoplasmic network with the spherules is clearly shown. The deeply stained portion of the nucleus is the vegetative chromatin, while the generative chromatins are distributed on linin.

Fig. 27. Part of a longitudinal section of *O. japonica*, stained as above, showing the form of pellicula as it appears in the cross section of Fig. 26.

Fig. 28. Part of a longitudinal section stained as above, showing however quite an abnormal colouration. The endoplasmic spherules which are stained in Figs. 26 and 27, do not take any colour in this case, while the ectosarc spherules which were not coloured in Figs. 26 and 27, are coloured very deeply black.

Fig. 29. Part of a tangential section of the body of *O. japonica*, showing the rows of basal granules of cilia, longitudinal ridges of pellicula, and very delicate transverse fibrils.

Fig. 30. Ectosarc spherules, showing the central clear spot, from an abnormally stained section, with HEIDENHAIN'S iron haematoxylin.

Fig. 31. Endoplasmic spherules, showing non-homogeneous state of the spherules. The peripheral portion of the granule is granulous, while the central portion is clear, within which however, very minute and faintly coloured granules are seen.

Fig. 32. Typical resting nucleus, showing the peripheral vegetative and the central generative chromatin.

Fig. 33. Nucleus, showing the extrusion of chromatin. Nearly half of the chromatin is seen to be extruded.

Fig. 34. Ditto: a mass of chromatin is extruded out of the nucleus and is seen lying in the cytoplasm.

Fig. 35. Normal resting nucleus.

Fig. 36. Early prophase nucleus. Peripheral vegetative chromatin shows the masses of various size, while the generative chromatins are seen gathered at the nodal portions of the linin.

Fig. 37. Prophase nucleus, a little more advanced. Masses of generative chromatin are united with each other and forming larger masses.

Fig. 38. Ditto: whole shape of the nucleus somewhat more elongated; the generative chromatin masses are gathered to form an incomplete spireme. The vegetative chromatin divided into many smaller masses.

Fig. 39. Middle prophase nucleus. It is now more elongated and shows the poles distinctly; the so-called spireme became thicker.

Fig. 40. Late prophase nucleus. Vegetative chromatin gathered in one large and two small masses; the loop of generative chromatin divides very irregularly and forms the chromosomes.

Fig. 41. Equatorial plate nucleus. Chromosomes show no regular arrangement.

Fig. 42. Early anaphase nucleus. Transverse division of the chromosome is now seen to occur.

Fig. 43. Ditto: shape of the nucleus is turned to a spindle in which vegetative chromatins are seen lying on the spindle fibers of one of its poles.

Figs. 44-46. Middle anaphase nuclei, showing the division or simply the separation of the chromosomes.

Figs. 47-49. Middle anaphase nuclei, slightly more advanced. Some of the chromosomes have already migrated to the poles, while the others are still at the equator or near it.

Figs. 50-52. Late anaphase nuclei. Migration of the chromosomes more advanced, and the connecting fibers are faintly to be recognized.

Fig. 53. Late anaphase stage, with the chromosomes placed in parallel position; the poles of the nucleus strongly pointed.

Fig. 54. Late anaphase stage.

Fig. 55. Early telophase nucleus; the median portion elongated.

Figs. 56-58. Early telophase nuclei, showing slight incurvings on both sides of the nuclei at the median portion.

Figs. 59-60. Middle telophase nuclei. The incurvings more increased and the nucleus bends at the median portion.

Fig. 61. Middle telophase stage.

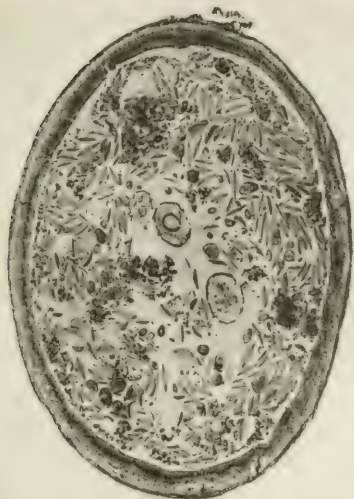
Figs. 62, 73. Late telophase nuclei. The median portions bulge out and the migrated chromosomes take more or less a parallel position.

Figs. 64-66. Late telophase. Migrated chromosomes become less dense and break up irregularly; the connecting strands thin and sometimes (Fig. 66) bent.

Fig. 67. Late telophase. The connecting strands are still continuous between the two nuclei.

Figs. 68-70. Late telophase. The connecting strands break up at the median portion, and the chromosomes also break into small masses or granules.

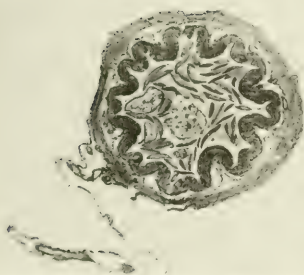
Fig. 71. Resting stage after division.



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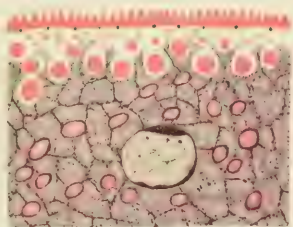
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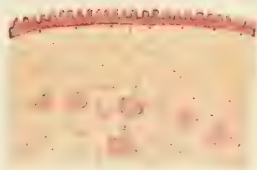
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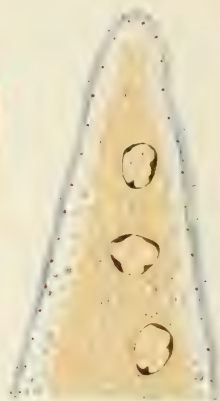
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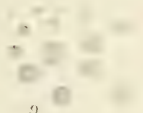
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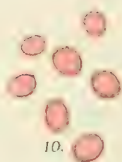
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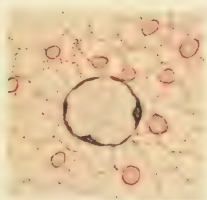
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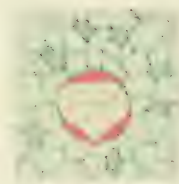
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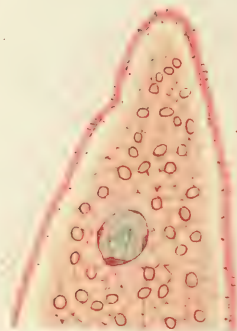
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11.



12.



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15.



16.



17.



18.



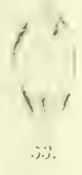
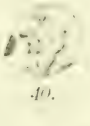
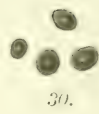
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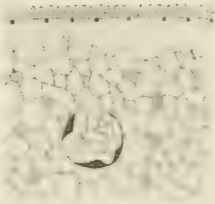


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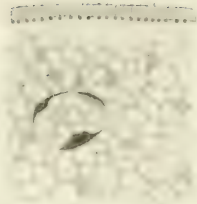


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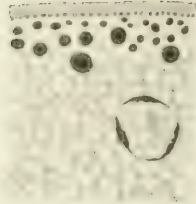




26.



27.



28.



32.



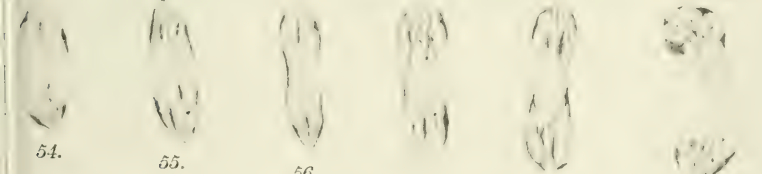
33.



34.



41. 42. 43. 44. 45. 46. 47.



54. 55. 56. 57. 58. 59.



65. 66. 67. 68. 69.



70. 71.

On the Inheritance of Flower-Colour and other Characters in *Digitalis purpurea*.¹

By

Kiichi Miyake and Yoshitaka Imai.

With Plate XLIII.

The experiments were started in 1916 in order to investigate the mode of inheritance of flower-colour and other characters in *Digitalis purpurea*. Although the experiments are still in progress, owing to the biennial nature of the plant it will take several years more before they can be completed, some of the results so far obtained do not agree with those of previous investigators. It seems desirable, therefore, to publish the results already obtained as a sort of preliminary report.

In May, 1916 we selected four plants designated as *A*, *B*, *C* and *D* in the accompanying table, which were growing in the College garden, and made crossings between them as well as self-pollination. In the following year four more plants were added to our experiments, which are marked *E*,² *F*, *G* and *H* in the table.

The seeds obtained by both self- and cross-fertilization of the above mentioned eight plants were sown during the autumn in pots filled with soil free from *Digitalis* seeds, and the young plants thus raised were transplanted into the experimental garden in the following spring. The offspring of *A*, *B*, *C* and *D* flowered in 1918 and 1919 while those of *E*, *F*, *G* and *H* bloomed mostly in 1919, some of them still remaining in rosette condition.

1. The substance of this paper was published in Japanese in the Botanical Magazine, Tokyo, vol. 33, pp. 175-186, 1919.

2. The plant *E* was secured through the kindness of our gardener Mr. M. Andô and is said to be the only surviving individual of F_1 plants obtained by crossing purple and white flowered plants in 1915.

The flowers of *Digitalis purpurea* being protandrous, pollination is usually effected by means of insects. Many cultivated *Digitalis* plants are therefore heterozygous for one Mendelian factor or more and do not breed true when self-fertilized. The majority of the plants used in the present experiments were also heterozygous and in fact none of the purple-flowered individuals were found to be pure, as is shown in the table. We shall, however, be able to report in a future paper on the results of crossings made with homozygous individuals.

Smooth or Hairy Stem.

Among the plants used in these experiments we have noticed the existence of two types in regard to the surface characters of the stem. One has the whole length of the stem covered with hairs, giving it a downy appearance, while in the other the vegetative lower region of the stem is quite glabrous. The genetic behaviour of these two characters has been considered worthy of investigation.

Miss SAUNDERS (1911) has already noted these two stem-characters in *Digitalis* and in a recent paper (1918) she conclusively proved that this partially glabrous condition of the stem is dominant to the hairy state. The results of our experiments can only confirm the excellent account of Miss SAUNDERS.

Of eight individuals used in the experiments six of them, namely the plants *A*, *B*, *C*, *F*, *G* and *H* had smooth stems and when selfed they have all excepting *B*¹ segregated into smooth and hairy forms. So we can see that these six plants are all heterozygous in regard to this character. The results of selfs² as well as crosses³ of the above mentioned plants are summarized as follows:

	Smooth stem	Hairy stem	Total
Obtained	267	92	359
Expected	269.25	89.75	359

1. Self-fertilized seeds of plant *B* were unfortunately lost, but its genetic constitution can well be inferred by the cross-bred offspring.

2. Families 1, 3, 8, 9 and 10 of the table.

3. Families 6, 11, 12 and 13 of the table.

Thus the ratio of the smooth-stemmed to the hairy is almost 3 to 1.

Our plants *D* and *E* were both hairy and when selfed they were found to breed true to type. Thus we have obtained 311 individuals which were all hairy.

When the smooth heterozygous individuals are crossed with hairy forms, we should expect to get smooth and hairy forms in equal numbers. The results of such crossing (Family 5 of the table) are:

	Smooth stem	Hairy stem	Total
Obtained	16	17	33
Expected	16.5	16.5	33

Although the numbers obtained are rather small they coincide nearly with those expected.

Thus the hairiness of the stem in *Digitalis purpurea* behaves as a simple Mendelian recessive to the smoothness. In many other plants, however, the hairiness is often a dominant character. In the smooth-stemmed *Digitalis* we may consider that it carries a factor which suppresses the formation of hairs in certain parts of the stem.

Flower-Colour.

The flower-colours concerned in the present experiments are:—

1. Purple with red spots (Plate XLIII, Fig. 1).
2. White with red spots (Fig. 3).
3. White with yellow spots¹ (Fig. 2).

Thus in all *Digitalis* flowers, whether white or coloured, the lower lip of the corolla is spotted, the spots being of various sizes. The number of spots also varies from only a few to several hundreds. In some of the last named white flowers the yellow spots turn brown as the flowers grow older, while in others the spots fade soon after the flowers are opened and become so faint as to be hardly distinguishable. In both purple and white flowers with red spots, the anthers are always speckled with red dots (Fig. 1c and 3c).

1. Although the colours of the spots, strictly speaking, are deep purplish red and yellowish green respectively, for the sake of brevity, we shall call them simply red and yellow.

Reference number of family	Designation	Parents		Offspring						Plants in rosette
				Coloured				Green		
		Character	Probable genetic constitution	Purple flower	White flower	White flower	White flower	Total		
				Smooth	Hairy	Smooth	Hairy	Smooth	Hairy	
1	A Self	C.R., P.F., S. St.	CcPP	4	1	—	—	2	2	9
2	B Self	C.R., P.F., S. St.	CcPP or CcPp	Lost
3	C Self	G.R., W.F., S. St.	ccPP (?)	—	—	—	—	8	2	10
4	D Self	G.R., W.F., H. St.	ccPP or cCPp	—	—	—	—	—	42	42
5 { ^a b	A × D	3	3	—	—	6	3	15
	D × A	1	5	—	—	6	6	18
6	B × C	24	14	—	—	34	14	86
7	E Self	C.R., W.F., H. St.	CcPP	—	—	—	209	—	60	269
8	F Self	C.R., P.F., S. St.	CcPp	61	12	1	2	19	8	103
9	G Self	C.R., P.F., S. St.	CcPP	17	6	6	1	—	—	30
10	H Self	C.R., W.F., S. St.	ccpp	—	—	—	—	11	2	13
11 { ^a b	F × G	13	5	1	1	—	—	20
	G × F	18	2	2	1	—	—	23
12 { ^a b	F × H	3	1	2	2	1	1	10
	H × F	3	2	7	2	5	1	20
13 { ^a b	G × H	8	5	7	1	—	—	21
	H × G	4	2	6	2	—	—	14
										14
										3 C.R.

C.R. = Coloured rosette.
G.R. = Green rosette.P.F. = Purple flower.
W.F. = White flower.S.St. = Smooth stem.
H.St. = Hairy stem.

According to KEEBLE, PELLEW and JONES (1910) the following allelomorphs are responsible for flower-colour:—

Mm; **M** being magenta colour factor, dominant to **m**.

Dd; **D** being a darkening factor dominant to **d** and converting magenta to purple.

Ww; **W** being a dominant white factor in the presence of which the expression of colour due to **M** is inhibited so that the flowers are white.

These authors considered that the white flowers with red spots, as in *Primula sinensis* (BATESON 1909, KEEBLE and PELLEW 1911, GREGORY 1912) carry a dominant white factor (**W**), which inhibits the expression of colour factor **M** except in the spots. The same opinion seems also to be held by Miss SAUNDERS (1911).¹ We have, however, reached a different conclusion, *i. e.*, all white flowers are recessive to the coloured and in no case have we found any evidence of the presence of a dominant white factor in *Digitalis purpurea*.

Among the coloured flowers almost every shade could be found between deep purplish red and very faint magenta. KEEBLE and his collaborators assume the intensifying factor **D** which converts light magenta to deep purple. In the present paper we shall not deal with the question of intensity of shade in the coloured flowers.

In both purple-flowered and red-spotted white-flowered plants, the stems and petioles are more or less purplish red, while in yellow-spotted whites they are entirely destitute of reddish tint and are quite green.

Among five coloured plants used, plants *A*, *E* and *F* segregated into coloured and green forms in the self-fertilized offspring, and plant *B* would probably have done the same if the self-fertilized seeds had not been lost, while plant *G* bred true to the coloured type. The results of self-fertilization of the former three individuals (Family 1, 7 and 8 of the table) are summarized as follows:

1. Without commenting on the dominant white factor she states:—"The observations made in regard to the inheritance of flower-colour are in entire accord, so far as they go, with the facts already published last year by KEEBLE, PELLEW and JONES."

	Coloured	Green	Total
Obtained	401	121	522
Expected	391.5	130.5	522

Thus the ratio of coloured and green being nearly 3:1, it agrees to the simple Mendelian expectation. From selfed *G* plant we have obtained 35 individuals which were all coloured.

When the heterozygous coloured plants are crossed with green individuals the ratio of coloured and green offspring should be 1:1. The results of such crosses (Families 5, 6 and 12 of the table) are summarized thus:

	Coloured	Green	Total
Obtained	82	85	167
Expected	83.5	83.5	167

If, however, heterozygous coloured plants are crossed with homozygous coloured plants we should expect the offspring all to be coloured. We have made such crosses (Family 11 of the table) and have obtained 44 coloured individuals.

When the coloured homozygote is crossed with a green individual the next generation should also be all coloured. We have obtained 43 plants as the result of such crosses (Family 13 of the table) and these were all coloured as expected.

Green forms, on the other hand, were found always to breed true to type. We have obtained 72 offspring by selfing three green plants *C*, *D* and *H*, and they were all green.

From the above mentioned experiments we may conclude that the green forms are recessive to the coloured and follow the simple Mendelian inheritance.

Coloured plants produce either purple flowers or white flowers with red spots, while green plants always have white flowers with yellow spots. The coloured plant *F* with purple flowers, when selfed, segregated into coloured and green forms, and the former produced both purple flowers and white flowers with red spots. Plant *G*, also with purple flowers, on the other hand, when selfed, yielded only coloured offspring. The flowers, however, were of two types, *i.e.*, purple, and white with red spots. The numbers obtained (Family 9) are:

	Purple flower	White flower	Total
Obtained	23	7	30
Expected	22.5	7.5	30

Thus the ratio of purple-flowered plants to white-flowered is approximately 3:1.

Plant *E*, with white flowers and red spots, when selfed, gave an F_1 composed of white flowers with red spots and white flowers with yellow spots, approximately 3:1 in ratio.

From the above mentioned facts and the results which will be described in the following pages (Families 8, 11, 12 and 13) we may conclude that purple flowers with red spots are dominant to white flowers with red spots.

For explaining the inheritance of colour, especially in flowers, we assume the following two allelomorphs:

Cc; **C** is a colour factor which is responsible for a reddish purple colour in stem, petiole, spots of the corolla and dots on the anther, dominant to **c**. **C** is absent from white flowers with yellow spots, which contain only **c**.

Pp; **P** being a factor in the presence of which the flower turns purple, dominant to **p**, is effective only in the presence of **C**. **P** is absent from white flowers with red spots which contain **C**.

The probable genetic constitution of plant *A* is **CcPP**, and when selfed, we should expect to get purples, and yellow-spotted whites in a 3:1 ratio. Observed numbers are, however, 5 of the former to 4 of the latter. This marked deviation is probably due to the insufficiency in numbers of F_1 raised. Plant *D* is assumed to be either **ccPP** or **ccPp**, and when crossed with *A*, we should expect to get purples and yellow-spotted whites in equal numbers. The numbers actually obtained are 12 of the former and 21 of the latter. Although unfortunately we lost the selfed seeds of plant *B*, when crossed with plant *C* we have obtained 38 purple-flowered plants and 48 whites with yellow spots. The genetic constitution of *B* would be **CcPP** (or **CcPp** in case *C* is **ccPP**), and that of *C* would be either **ccPP** or **ccPp**. Thus in F_1 of the cross $B \times C$ we should expect to have purples and whites with yellow spots in equal numbers.

When plant *E*, white with red spots, was selfed we obtained in the

subsequent generation 209 whites with red spots and 60 whites with yellow spots. We have also 127 F_1 plants still in rosette of which 100 are coloured and 27 are green. The former will no doubt produce white flowers with red spots and the latter white flowers with yellow spots. Summing up the above we have:

	White with red spots	White with yellow spots	Total
Obtained	309	87	396
Expected	297	99	396

We may, therefore, conclude that the genetic constitution of plant *E* would be **Cc \bar{c} pp**. Thus these two kinds of white flowers would be approximately in a 3:1 ratio, with their probable genetic constitutions:

$$\begin{array}{rcl}
 1 \text{ CCpp} : & 2 \text{ Cc} \bar{c} \text{pp} : & 1 \text{ ccpp} \\
 \hline
 3 \text{ white with red spots} : & & 1 \text{ white with yellow spots.}
 \end{array}$$

Plant *F*, with purple flowers, when selfed gave an F_1 composed of 73 purples, 3 whites with red spots, and 27 whites with yellow spots. The probable genetic constitution of plant *F* being **CcPp**, we should expect to have the three kinds of plants in a 9:3:4 ratio, but numbers of whites with red spots actually obtained are below this ratio. We have, however, 11 coloured and 3 green plants still in rosette condition, and if most of the coloured individuals are whites with red spots the deviation will be very much lessened.

When we have selfed plant *G*, the F_1 individuals have been composed of purple-flowered, and white-flowered with red spots approximately in a 3:1 ratio, the actual numbers being 23 to 7. So the genetic constitution of plant *G* is very likely **CCPp**.

We should expect F_1 of the cross $F \times G$ to yield purples, and whites with red spots in a 3:1 ratio. The numbers actually obtained in experiments were 38 of the former and 5 of the latter.

Plant *H*, white with yellow spots, when selfed gave 13 offspring all white with yellow spots. Thus plant *H* is homozygous in regard to factor *c*. When *H* has been crossed with *F*, we have obtained 9 purples, 13 whites with red spots, and 7 whites with yellow spots. As the genetic constitution of plant *F* is **CcPp**, that of *H* would be **ccpp**. We should

therefore expect to produce these three kinds of plants in a 1:1:2 ratio, but the numbers of white with yellow spots actually obtained were much smaller than this. There are, however, 8 green individuals among 18 rosette plants. As all green plants produce white flowers with yellow spots, by including these the expected number will be approached. By crossing plant *H* with plant *G*, we should expect to have purple-flowered individuals and whites with red spots in equal numbers. The numbers actually obtained were 19 and 16.

The results obtained by KEEBLE and his collaborators may be explained, in our opinion, in the following way. KEEBLE's plant *A*, with purple flowers, when selfed, yielded an F_1 composed of 17 purple-flowered plants and 5 whites with yellow spots. The genetic constitution of *A* is very likely **CcPP**. KEEBLE's *D* and *F* both had white flowers with red spots. When they were selfed, 11 whites with red spots, 2 whites with yellow spots and 1 purple were obtained from the former, while from the latter 4 whites with red spots and 1 purple were produced. Of these two purple-flowered plants, KEEBLE considered the latter a rogue, since this individual being non-peloric, can not be produced from peloric parent *F*, *p*-loria being recessive to normal. On the other hand, KEEBLE seems to lay great stress on the appearance of the first mentioned purple, regarding it as indicating the dominance of white with red spots. As has been mentioned by Miss SAUNDERS (1918), in experiments with *Digitalis* it is not uncommon to find rogue or stray plants. In our opinion the purple-flowered plant among the F_1 generation of KEEBLE's plant *D* was also a rogue. The genetic constitution of KEEBLE's plant *D* and *F* would thus be **CcPp** and **CCpp**.

KEEBLE's plant *E*, white with yellow spots, when selfed, has bred true. So the plant is homozygous in regard to factor *c*. By crossing *D* and *E*, KEEBLE obtained an F_1 composed of 10 purples, 11 whites with red spots, and 25 whites with yellow spots. It would seem to us that the genetic constitution of *E* is probably **ccPp**. By crossing with *D* we should then expect these three types of flowers in a 1:1:2 ratio:

$$\begin{array}{rcl} 1 \text{ CcPp} & : & 1 \text{ CcPp} & : & 1 \text{ ccPp} : 1 \text{ ccpp} \\ 1 \text{ purple} & : & 1 \text{ white w. red spots} & : & 2 \text{ white w. yellow spots.} \end{array}$$

According to our assumed genetic constitution, the cross $F \times E = CcPp \times ccPp$. So that F_1 will contain:—

$$2 CcPp : 2 Ccpp$$

2 Purple : 2 White with red spots.

Both purples and red-spotted whites should be produced in equal number. KEEBLE, however, has obtained 25 purples and 15 whites.

KEEBLE was not quite sure of the genetic constitution of his plant B. It would seem very likely to have been $CcPp$, and to belong to an extreme faint form of purple-flowered plant (described by KEEBLE as white with purple flush and red spots). Thus when B is selfed, we should expect to get an F_1 generation composed of purples, whites with red spots, and whites with yellow spots in a ratio of 9:3:4. Actual numbers obtained by KEEBLE were 6 purples, 2 whites with red spots and 4 whites with yellow spots.

KEEBLE's results are thus explained, without assuming the presence of a white dominant factor.

Summary.

1. Partial smoothness of the stem in *Digitalis purpurea* is a Mendelian recessive to hairiness, as has been shown by Miss SAUNDERS.

2. The allelomorphs responsible for colour are:—

Cc ; C being a colour factor, dominant to c .

Pp ; P being a factor which is active only in the presence of C and converting the flower-colour to purple.

3. In the presence of the colour factor C , the plant shows a varying purplish colour in stems, petioles and flowers, when this is absent the plant is quite green with white flowers, coloured being dominant to green.

4. When both factors C and P are present, the flowers are purple. Intensity of the purple hue varies much, from deep purplish red to a very faint magenta, but this is outside the scope of this paper.

5. When factor P is absent from plant containing factor C , the flowers are white with red spots. There is no proof that this type of flower is a dominant white.

6. In the absence of factor **C**, the flowers are white with yellow spots. In this type of white flower, there are genotypically two forms, one with factor **P** and the other without it.

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EXPLANATION OF PLATE LXIII.

Fig. 1. Purple flower.

- a. Entire flower.
- b. Lower lip of the corolla.
- c. Stamens.

Fig. 2. White flower with yellow spots.

- a. Entire flower.
- b. Lower lip of the corolla
- c. Stamens.

Fig. 3. White flower with red spots.

- a. Entire flower.
- b. Lower lip of the corolla
- c. Stamens.



a.



c.



b.

Fig. 1.



a.



c.



b.

Fig. 2.



a.



c.



b.

Fig. 3.

Der Farbensinn bei Fischen.

(Karpfen und Goldfische.)

VON

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Mit Tafel XLIV und 26 Textfiguren.

I. Historische Übersicht.

Über den Farbensinn bei Fischen sind schon zahlreiche Arbeiten erschienen, von denen alle, mit Ausnahme der von C. HESS, die Existenz eines Farbensinnes bestätigen. Die Geschichte dieser Forschungen können wir in zwei (oder besser in drei) Perioden einteilen.

DIE ERSTE PERIODE (— bis 1909).

Im Jahre 1884 untersuchte GRABER die Frage der Farbenunterscheidung bei zwei Arten, konnte aber zu keinem überzeugenden Resultate gelangen. BATESON (1887) wiederholte die Versuche mit farbigen Ziegeln, von denen weisse und blassblau leicht, aber blaue und dunkelrote schwer erkannt wurden. Er war auch der erste, der die Lichtintensität mit berücksichtigte. ZOLOTNITZKI (1901) liess die Fische ihr Futter, roten *Oleonoronotus*, mit weisser, grüner, gelber und roter Wolle vergleichen. Sie zogen die rote den anderen Farben vor, gingen an weisser und grüner vorüber, verweilten aber etwas vor der gelben. WASHBURN und BENTLEY (1908) gebrauchten eine Zange mit farbigem Merkmal anstatt BATESON's Ziegeln und machten Versuche bei *Senotilus atramaculatus*. Sie berichten: "Two dissecting forceps were used alike, except that to the legs of one were fastened, with rubber bands, two small sticks painted red, while to those of the other similar green sticks were

attached. The forceps were fastened to a wooden bar projecting from a wooden screen, which divided the circular tank into two compartments, and hung down into the water. Food was always placed in the red pair of forceps, which were made frequently to change places with the green ones; and the fishes were caused to enter the compartment half of the time on one side and half of the time on the other. This was to prevent identification of the food fork by its position or the direction in which the fish had to turn. The animals quickly learned to single out the red fork as the one important to its welfare, and in forty experiments, mingled with others so that the association might not be weakened, where there was no food in either fork, and where the forceps and rubber bands were changed so that no odor of the food could linger, it never failed to bite first at the red. Moreover, the probability that its discrimination was based upon brightness was greatly lessened by using, where we experimented without food, a different red much lighter than that in the food tests. The fish successfully discriminated red from blue paints in the same way, and it was afterwards trained, by putting food in the green fork, to break the earlier association and bite first at the green." REIGHARD (1908) 'gebrauchte *Lutjanus griseus* bei seinen Experimenten mit gefärbtem Futter, *Atherina*. Bei Diskrimination von weiß und blau sind sie positiv für weiss; von blau und hellrot, blau und dunkelrot, blau und gelb immer positiv für blau; aber blau mit grün verglichen suchen sie beide gleich oft. Auch verwerfen sie nach der Erziehung mit *Cassiopa* rot gefärbtes Futter.

Die leicht bemerkbare Ungenauigkeit in diesen Versuchen liegt in der unvariieren Lichtintensität, so dass bei der Diskrimination von verschiedenen Farben nicht klar wird, ob die Vorliebe der Fische für oder gegen die Farbe von dem Farbenton oder von der Lichtintensität abhängt. Obschon zwar WASHBURN und BENTLEY diesen Einfluss der Lichtintensität auszuschalten versuchten, so blieb doch zweifelhaft, ob das Rot nicht einen stärkeren Reiz ausübt, infolge seiner Intensität, verglichen mit Grau, Grün, Blau u. a. Spielt die Intensität bei diesen Experimenten auch eine Rolle, so entsteht die Frage: Reagieren die Fische vielleicht auf das Rot, weil es viel heller ist? Ich meine also, es wäre nötig gewesen zu versuchen, nicht nur, ob die Tiere zwischen verschieden graduiertem Rot und z. B. Grün unterscheiden, sondern

auch zwischen Rot und verschieden graduiertem Grau. Dies könnte vielleicht andere Resultate ergeben haben.

DIE ZWEITE PERIODE (1909—1911).

Diese Periode habe ich als Vorläufer der nächsten nur der Bequemlichkeit wegen aufgestellt. Sie ist gekennzeichnet durch den Streit zwischen V. BAUER und C. HESS, dem einzigen, der den Fischen einen Farbensinn überhaupt abgestritten hat. HESS (1909) untersuchte den Lichtsinn bei Fischen. Diese Arbeit ist mir noch nicht zu Gesicht gekommen, doch vermutete ich, dass seine damaligen Resultate in seiner späteren Arbeit mit verwendet worden sind. BAUER (1910) studierte *Ceharax puntazzo*, *Atherina hepsetus* L., *Box salpa* c. v. und *Mugil* sp. auf ihre spektrale Reaktion und schloß, daß „durch verschiedene Adaptationszustände Unterschiede in der Reaktion auf verschiedene Spektralfarben und farbige Glaslichter hervorgerufen werden, welche dafür sprechen, dass die Farben für die Fische außer ihrem Helligkeitswert noch einen (nur bei Hell-Fischen) Farbenwert besitzen,“ und das Hess'sche Phänomen (dass die Fische nur einen den dunkeladaptierten oder totalfarbenblinden Menschen ähnlichen Farbensinn haben) nur in dunkeladaptierten Fischen auftritt. Und auch: „Wie bei normalen Menschen tritt bei Helladaptation zur Unterscheidung der Helligkeit die Unterscheidung der Farbenwerte (Rotschen bei *Chorax*, *Atherina*; Vorliebe für Blau bei *Box*).“ Von diesem Standpunkt aus schließt er auf einen Farbensinn bei Fischen. C. HESS (1910) leugnete aber in seiner Arbeit „Über den angeblichen Nachweis von Farbensinn bei Fischen“ alle von BAUER dargestellten Ergebnisse und schrieb: „Aus den besprochenen Untersuchungen, die BAUER gegen mich anführt, kann also nur der eine Schluss gezogen werden, dass auch diese Fische sich offenbar ähnlich wie die von mir untersuchten und durchaus so verhalten, wie es der Fall sein muß, wenn ihre Sehqualitäten ähnlich oder die gleichen sind wie jene des totalfarbenblinden Menschen.“ Endlich sagte er, „dass keine von ihnen das Vorkommen von Farbensinn bei Fischen auch nur wahrscheinlich macht.“ BAUER (1910) behauptete in der Erwiderung seine vorige Ansicht.

DIE DRITTE PERIODE (1911—).

An Bauers Stelle trat jetzt V. FRISCH auf; die zwischen ihm und C. HESS bestehende Meinungsverschiedenheit wurde in den folgenden Jahren oft erörtert und ihre Ergebnisse waren wesentlich. Zur Übersicht führe ich im folgenden die Hauptpunkte des Streites an.

1. Die HESS'sche Behauptung.—Sie stützt sich im wesentlichen auf zwei Hauptpunkte: eine spektrale Untersuchung und eine andere Vergleichungsmethode.

„Stellt man ein Bassin mit Jungfischen, die eine halbe Stunde oder länger dunkel gehalten waren, so auf, dass die Breite des Spektrums ungefähr der Breite des Bassins entspricht, also dessen verschiedene Teile von verschiedenen homogenen Lichtern durchstrahlt werden, so schwimmen bald fast alle Tiere in der Richtung gegen das Gelbgrün bis Grün des Spektrums: in wenigen Sekunden hat sich die große Mehrzahl der Fische hier gesammelt. Schon im Gelb sind sie jetzt wesentlich spärlicher, nach dem Gelbrot nimmt ihre Zahl rasch beträchtlich ab, und im Rot des Spektrums bleiben im allgemeinen wenige oder gar keine Fische, ebenso in den dunklen, dem Ultrarot entsprechenden Partien des Bassins. Auch nach der anderen Seite des Grün, gegen das kurzwellige Ende des Spektrums zu, nimmt die Zahl der Fische beträchtlich, doch nicht so rasch ab, wie gegen das langwellige Ende; im Grünblau bis Blau bleibt meist noch eine, freilich nicht große Zahl von Fischen, die gegen das Violett hin immer kleiner wird“; und „verschiebt man durch eine entsprechende kleine Bewegung der großen Konvexlinse vor dem Prisma das Spektrum um einige Zentimeter nach links, so haben sich in wenigen Sekunden nahezu sämtliche Fischchen nach links gedreht und schwimmen, grösstenteils parallel zueinander, wieder der Gegend des Grün zu; wird das Spektrum nun rasch in der entgegengesetzten Richtung verschoben, so macht die ganze Schar kehrt und eilt wieder zum Grün.“ Von dieser Beobachtung ausgehend, bemühte er sich eine weitere „messende“ Untersuchung anzustellen, die folgendes ergab: „Für die in Rede stehenden See- und Süßwasserfische liegt die hellste Stelle des Spektrums in der Gegend des Gelbgrün bis Grün. Die Helligkeit nimmt für sie von hier gegen das langwellige Ende rasch ab und ist schon in der Gegend des Gelb

wesentlich kleiner als in der Gegend des Gelbgrün bis Grün; die gelbroten und roten Strahlen des Spektrums haben für diese Fische nur einen sehr kleinen Helligkeitswert. Nach dem kurzwelligen Ende von der Gegend des Gelbgrün bis Grün nimmt gleichfalls die Helligkeit des Spektrums für sie ab, doch weniger rasch als nach dem langwelligen.“ D. h., „die Kurve der relativen Helligkeiten zeigt also auch hier die für das totalfarbenblinde Menschenauge charakteristischen Eigentümlichkeiten.“ Aus den bisher mitgeteilten Untersuchungen schließt er: „Die Fische verhielten sich also bei allen meinen Untersuchungen annähernd (oder genau) so, wie totalfarbenblinde Menschen bei jeder und wie normale dunkeladaptierte Menschen bei herabgesetzter Lichtstärke sich verhalten würden, die unter entsprechende Verhältnisse gebracht, nach den jeweils für sie hellsten Stellen streben.“

Nächst will ich seine zweite Untersuchung übersichtlich schildern, die „Gleichung“ zwischen farbiger Attrappe und farbigem oder farblosem Grund. Der Kürze wegen will ich nur seinen Schluss darstellen und nicht den Prozess, „daß die Fische auf die Attrappe oft losfuhren, wenn ihr farbloser Helligkeitwert von dem des Grundes wesentlich verschieden war, wie immer die Farben beider uns erscheinen möchten; daß sie dagegen, wenn der farblose Helligkeitwert der Attrappe von jenem des Grundes nur wenig oder gar nicht verschieden war, ihr wenig oder gar keine Beachtung schenkten, auch dann, wenn sie für unser Auge sich durch lebhafte Färbung aufs deutlichste vom Grunde abhob.“ Um weiter seine Versuche mit möglichst freifarbigen und zugleich kontinuierlich variablen Reizlichtern anzustellen, bediente er sich eines Verfahrens, dessen modifizierter Apparat der meinige ist, und schließt aus diesem Versuche: „Alle diese Befunde bleiben unverstänlich, wenn man den untersuchten Fischen Farbensinn zuschreiben will; sie sind ohne weiteres verständlich, ja zu erwarten, wenn die Sehqualitäten der Fische ähmliche oder die gleichen sind wie die des totalfarbenblinden Menschen.“

Seine Untersuchung ist am meisten wissenschaftlich, wie er selbst sagte. Wie verschieden auch andere, von anderen Autoren aufgestellte Ergebnisse sein mögen, so werden die seinigen immer bestehen bleiben, insofern niemand eine entgegengesetzte Meinung aufstellen kann, welche noch nicht von ihm selbst schon einmal versucht worden ist.

Eine Frage, welche ich, ein Laie, bezüglich seiner (ophtalmologischen)

Spektraluntersuchung liegt, ist die Helligkeit seines Spektrums, das auf die jungen Fische im Aquarium fiel. Bei skotopischen Menschen wird der hellste Punkt in der Luminositätskurve vom Spektrum nach der kurzwelligen Seite geschoben; das findet sich auch gleicherweise im dunkeln Spektrum bei photopischen Menschen (Fig. 1, 2 und 3). Wenn das von Hrn. Hess in seiner Untersuchung angewandte Spektrum das Dunkelspektrum (nur in dieser Grenze in seiner Helligkeit kontrollierte) war, so musste er notwendig eine den Totalfarbenblinden ähnliche Luminositätskurve gewinnen, obwohl die Fische gar nicht farbenblind waren. Die Helligkeit des in seiner Untersuchung gebrauchten Spektrums ist also durchaus von Belang.

2. v. FRISCH'S Behauptung.—Seine Bestätigung des Farbensinns bei Fischen gründet sich auf die Untersuchung der Anpassung bei Fischen am Grund: „Roter und gelber Untergrund veranlassen genau die gleiche Farbanpassung. An grünen, blauen und violetten passen sich die Pfrillen nur ihrer Helligkeit an.“ Dagegen „bei blinden Pfrillen bleibt selbst monatelange Haltung in farbigem Lichte völlig ohne Einfluss auf den Expansionszustand der Pigmentzellen, und auch ein Einfluss der Farben auf die Pigmentbildung war nicht nachzuweisen.“ In seinen vielen Arbeiten behauptet er immer diese farbige Anpassung an den Boden, aus der er den Farbensinn bei Fischen glaublich machen will.

Was zugleich ihn zu diesem Schluß leitet, ist die Farbenänderung der Fische in ihren Sexualzeiten, die er als ein Hochzeitskleid bezeichnet, welches bestimmt sei, das andere Geschlecht anzuziehen, und so bejaht er teleologisch den Farbensinn bei Fischen.

Die Hauptteile ihrer beiderseitigen Behauptungen wurden oben abgekürzt, aber wenn man die von jedem von ihnen erhaltenen Ergebnisse vergleicht, so findet man ganz und gar keine Übereinstimmung. v. FRISCH schrieb, bei den mit gelben Futter gefütterten Fischen positive Diskrimination zwischen gelben und grauen, fast gleichhellen Attrappen gesehen zu haben; dagegen sagte C. HESS, dass bei dem Anpassungsphänomen keine Verschiedenheit zwischen rotem und grauem Grund beobachtet werde, und auch dass die Fische ihre Hochzeitskleider in den tieferen Wasserschichten wohl nicht erkennen können, wie wir sie in der Luft sehen, „unsere Fische sich rot färben, um gelb auszusehen?“

Dies ist die historische Übersicht in bezug auf den Farbensinn bei Fischen. Diese Aufgabe bleibt also nach drei Richtungen hin noch weiter zu untersuchen: spektrale Versuche, Vergleichung der Objekte mit dem Grund und das Anpassungsphänomen an den farbigen Boden.

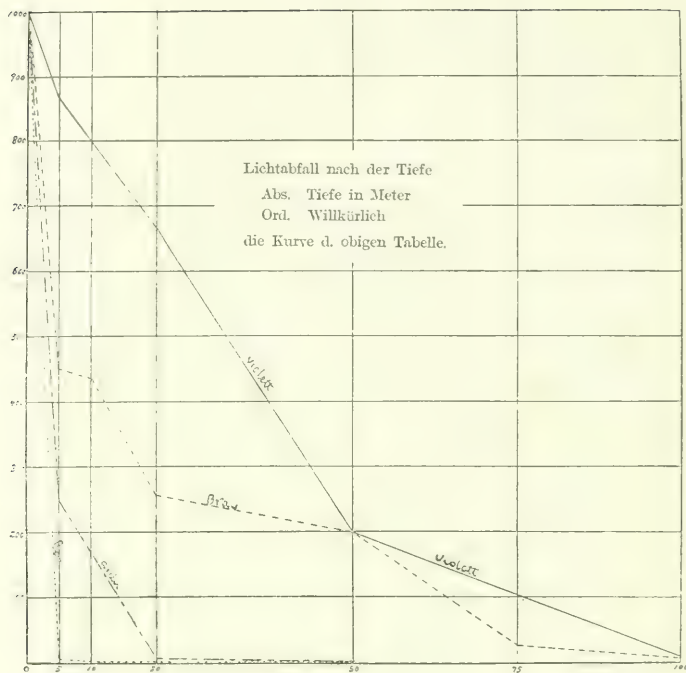
Weiter will ich etwas näheres über die Hochzeitsfarbe, welche von v. FRISCH und anderen als ein Beweis für den Farbensinn angeführt wird, schreiben, bevor ich dieses Kapitel zu Ende bringe.

Die verschiedene Absorption des Wassers von verschiedenen Lichtstrahlen ist eine auffallende, schon bereits bekannte Tatsache; besonders rotes und orange-gelbes Licht wird bald nach dem Eindringen ins Wasser sehr schnell absorbiert, tiefer als 5 m finden sich nur 0.37% und 0.25% der in 1 m Tiefe vorhandenen Lichtmenge; während grüne und andere kurzwellige Strahlen nicht so stark absorbiert werden, weshalb der Farbenton des Wassers da blau aussieht, und die durch die Wasserschicht gesehene Farbe wird ihren roten Ton verlieren und einen neuen, blauen Ton annehmen.

TABELLE I.

Lichtabfall nach der Tiefe zu, ausgedrückt in Tausendteilen
der in 1 meter vorhandenen Lichtmenge.

Tiefe m.	Rot.	Orange-gelb.	Grün.	Blau-grün	Blau.	Blau-violett.
1	1000.	1000.	1000.	1000.	1000.	1000.
5	3.7	2.5	250	250	450	866
10	2.7	2.0	166	166	437	800
20	0.03	1.2	5.8	21	277	666
50	0.0021	0.032	2.2	2.5	201	200
75		0.008	0.75	2.2	25.6	100
100		0.001	0.03	0.033	5.5	10
200			0.004	0.01	0.04	1.
500			0.0016	0.004	0.004	0.1
1000				0.0003	0.0001	0.003
1500						(0.00001)



Textfigur 1.

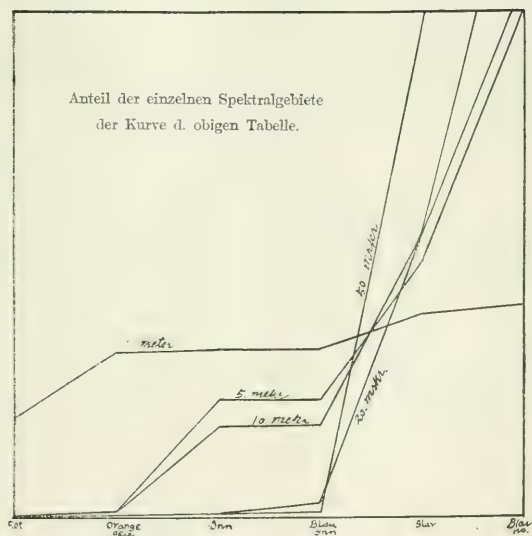
TABELLE II.

Anteil der einzelnen Spektralgebiete an der Zusammensetzung
des Lichtes in den verschiedenen Tiefen.

Gesamtlichtmenge in jeder Tiefe = 1000.

Tiefe m.	Rot.	Orangegelb.	Grün.	Blau-grün.	Blau.	Blau-violett.
1	96.7	165.7	165.7	165.7	198.9	207.3
5	0.98	1.18	117.3	117.3	254.0	508.8

Tiefe m.	Rot.	Orangegeb.	Grün.	Blau-grün.	Blau.	Blau-violett.
10	0.84	1.06	89.64	89.64	282.3	537.1
20	0.018	1.05	4.68	17.26	279.71	697.2
50	0.0025	0.069	4.53	5.04	486.0	504.0
75		0.054	4.73	14.20	193.6	787.5
100		0.0052	1.56	1.73	346.2	650.8
200			3.18	8.06	37.16	952.0
500			12.27	30.63	30.80	920.3
1000				74.69	37.31	888.1
1500						(1000.0)



Textfigur 2.

„Bereits in Tiefen von nur 3—4 m erscheint ein in der Luft leuchtend roter Fisch, auch wenn das Licht nur schräge, nicht senkrecht, nach unten gerichtet ist, nicht mehr rot, sondern im allgemeinen nur braungrau bis

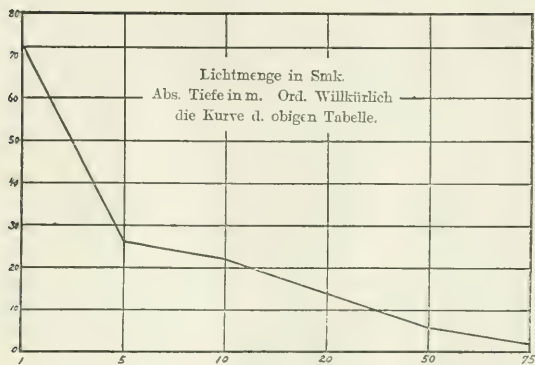
gelbgrau, bei günstiger Beleuchtung schwach rötlich graugelb.“ So schrieb C. Hess von seinem wirklichen Experiment. In einer Tiefe von 50 m beträgt das rote Licht nur 2,1 und orangegelbes 32 in Millionteilen, selbst grünes nur 2,2 und das blaugrüne 2,5 in Tausendteilen des Lichts an der Oberfläche; während blaues und blauviolett in dieser Tiefe noch hunderttausendmal stärker bleiben als rotes. Daher können dort laichende Fische nicht mehr die Hochzeitsfarben, und besonders nicht die langwelligen, erkennen, wie wir sie in der Luft sehen. Besonders ist die Frisch'sche Ansicht, in dieser Hochzeitsfarbe ein Anziehungsmittel zu vermuten, undenkbar bei den in der Nacht laichenden.

Je tiefer wir ins Wasser hinabtauchen, desto dunkler wird es, das Wasser absorbiert nämlich die verschiedenen Lichtstrahlen allesamt, und die Helligkeit fällt so schnell ab, daß in 50 m Tiefe nur $1/12$ der in 1 m Tiefe vorhandenen Lichtmenge dringt; also in dieser dunklen Gegend haben sie vielleicht monochromatische Augen, die wir normalen Menschen in der Dämmerung besitzen.

TABELLE III.

Lichtmengen in Smk. (Sekunden-meter-kerzen.)

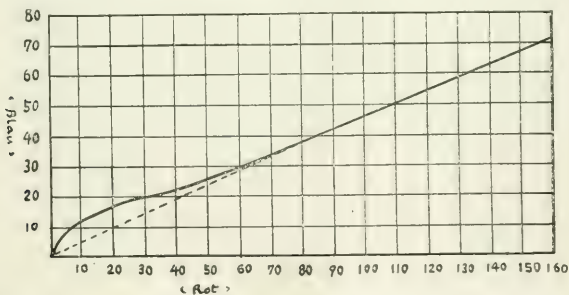
Tiefe m.	Rot. 680—610	Orange-gelb. 620—585	grün. 570—515	Blaugrün. 545—486	Blau. 475—420	Blau-violett. 435—400
1	700	1200	1200	1200	1440	1500 Smk.
5	2.5	3	300	300	648	1300
10	1.89	2.4	200	200	630	1200
20	0.027	1.5	7	25.2	400	1000
50	0.0015	0.041	2.7	3.	290	300
75	—	0.0104	0.9	2.7	37	150
100	—	0.0012	0.036	0.04	8	15
200	—	—	0.005	0.013	0.06	1.5
500	—	—	0.002	0.005	0.006	0.15
1000	—	—	—	0.0004	0.0002	0.005
1500	—	—	—	—	—	(0.00002)



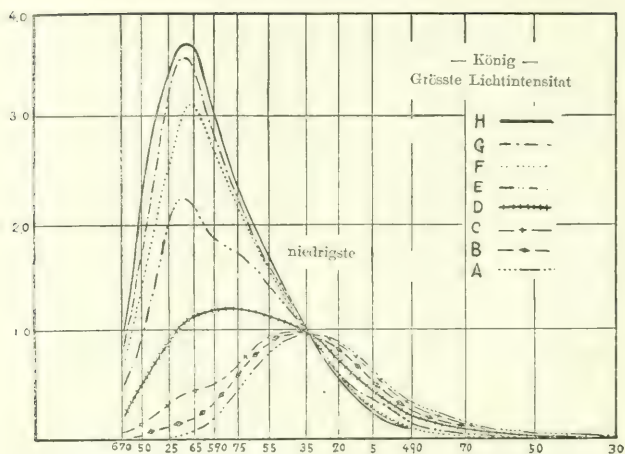
Textfigur 3.

Wenn die Fische auch wesentlich nicht farbenblind sind, werden sie in solchen Tiefen wirklich farbenblind. Obschon nicht monochromatisch, hindert wenigstens die Abkürzung des Spektrums an der langwelligen Seite bei Skotopischen die Diskriminationsfähigkeit der Fische, besonders die von Rot (vergl. Fig. 1—3).

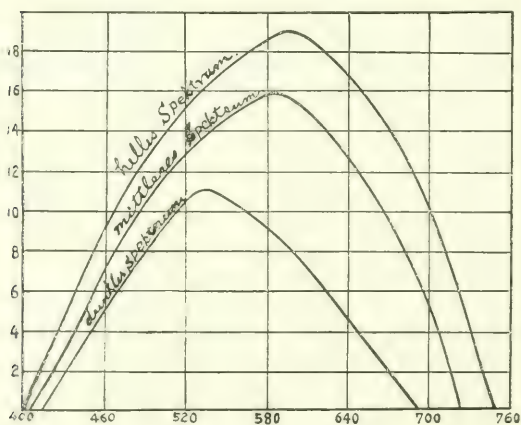
Dazu kommt noch Purkinjes Phänomen, welches C. HESS bei seinen Fischen leugnet, aber seine Gegner behaupten. Jedoch meine ich, dass dieses Phänomen, wenn es sich findet, sie getäuscht hat, weil die Luminosität des Rots, wie die Kurve (Fig. 4) zeigt, niedriger als die des Blaus wird, gemäss der totalen Luminositätsabnahme.



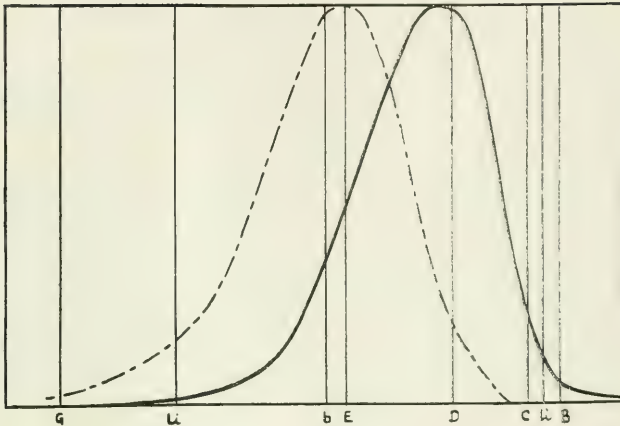
Textfigur 4. Kurve der gleichen Luminosität des Rot- (670) und Blaulichtes (450) bei verschiedener Intensität. (Schlitzweite) (Nach Köster)



Textfigur 5. Normale trichromatische Luminositätskurve für verschiedene Lichtstärke.
Abscissen: λ des prismatischen Spektrums des Gaslichts.
Ordinaten: willkürliche Skala. (Nach König.)



Textfigur 6. Luminositätskurve (Nach HAYCRAFT)



Textfigur 7. Normale trichromatische photopische und skotopische Luminositätskurve.

Eine Amylacetat- oder Hefner-Einheit ist gleich 0,9 internationaler Kerzenstärke.

---, Luminosität des Spektrums für ein normales Auge, das Tageslicht reduziert auf $\frac{1}{132,5}$ einer Amylacetalampe 1 Fuss vom Schirm entfernt; —, Luminosität für das normale Auge mit gewöhnlicher Intensität des Spektrums.

Luminosität für das normale Auge mit gewöhnlicher Intensität des Spektrums.

Also in dunkler Tiefe können die Fische, wenn sie auch trichromatisch, wie behauptet, vergleichend das Blau wohl sehen, aber das Rot (langwellige Strahlen, d. h. die Farbe des Hochzeitleides) nicht so wohl sehen können.

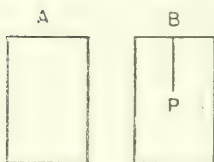
Demnach ist es sehr zu bezweifeln, dass die Fische an ihrem Wohn- oder Laichplatze, wenn dieser nicht sehr seicht ist, ihre prächtigen Hochzeitskleider so sehen können, wie wir es in der Luft instande sind, und Frisch's Ansicht kann demnach kaum die richtige sein.

Sind dagegen die Fische monochromatisch, so ist das Anpassungsphänomen immer noch zu erklären, weil Monochromaten die Farbe nach ihrer Helligkeit unterscheiden, wenn auch nicht die Farben selbst. Innerhin lässt die Frage des Hochzeitskleides noch einige Zweifel zu. Wenn meine Vermutung richtig ist, ist es eine physiko-chemische (Hormonen-) Erscheinung, die mit dem körperlichen Zustande ohne Beziehung auf den Farbensinn auftreten muss.

II. Apparat und Strahlenfilter.

Wie der Schall drei Eigenschaften: Intensität (loudness), Höhe (pitch) und Ton (timbre) hat, so auch die Farbe drei: Ton, Intensität und Sättigung; lassen wir einen Punkt aus, so kommen wir der Wahrheit nicht nahe. Besonders ist die Farbenuntersuchung ohne Beachtung der Intensität, sozusagen, eine Luftspiegelung. Doch ist sie am schwierigsten zwischen zwei Farben, orzüglich verschiedenen Farben, zu vergleichen, und est nicht leicht zu bestimmen, welche von ihnen mehr dunkel oder hell ist. Zu diesem Zweck giebt es eine flackernde Methode, die ziemlich richtige Ergebnisse liefert, aber von niemand bisher vielleicht wegen ihrer Schweranwendbarkeit in den Versuchen gebraucht worden ist.

I. EINKAMMER-AQUARIUM.



Textfigur 8.
Erklärung im Text.

Ich benutzte ein Einkammer-Aquarium (Fig. 8, A) das in der Diskrimination des zu untersuchenden Lichtes, besonders bei Fischen, in einigen Punkten besser ist, als das bisher in Experimenten auf diesem Gebiete meist gebrauchten Zweikammer-Aquarium (Fig. 8, B). Die Tiere können immer, nicht verhindert von der Trennung (P), herumschwimmen und ihre Absichten werden früher und dentlicher beobachtet, eine notwendige Bedingung bei diesen Vergleichungsversuchen.

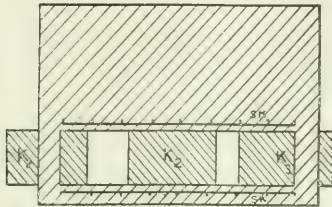


Textfigur 9.
Erklärung im Text.

Die zwei Längswände (Fig. 9) sind durch schwarze Kartons vor seitlich einfallendem Lichte geschützt und die übrigen zwei bleiben frei, deren eine (Fig. 9, c. d.) aber beliebig durch den Schirm (S) geschützt wird, an dem ein Ausschnitt (A), durch den die Strahlen einfallen, sich befindet. Auch die a b Seite kann beliebig geschützt werden.

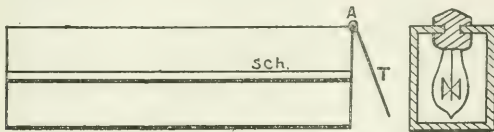
2. DER SCHIRM. (Textfig. 10).

Mit den Kartons, K_1 , K_2 , u. K_3 , wird die Weite des Ausschnitts beliebig kontrolliert, die die Skala (sk) zeigt.



Textfigur 10.

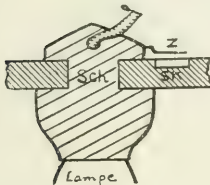
Kasten besteht aus drei Teilen: Skalakasten, bewegliche Dille (Lampe) und Überdicke.



Textfigur 11.

i) Skalakasten (Textfig. 11):—Aus einem Holzbrett gebildet, hinten eine Tür (T) mit Angel (A) versehen, das Lampenlicht kommt aus dem Vorderteil.

Oben hat es einen Längsschnitt (Sch) mit Skala (Sk), dem entlang die bewegliche Dille geschoben wird. Skala in Zentimetern. Innenwände schwarz.



Textfigur. 12.

ii) Bewegliche Dille (Textfig. 12):—Sie hat in der Mitte einen Zeiger (z), welcher die Stellung der Lampe, ihre Entfernung von der Aquariumwand, in cm zeigt. Mit beliebiger Kerzenlampe versehbar.

iii) Überdicke:—Das durch den Ausschnitt (Sch) fließende Licht abzuhalten, sonst können wir die Lichtintensität an der Wand des Aquariums nicht bestimmen.

Dieser Kasten ist aber nur eine ganz einfache Vorrichtung, welche ich selbst konstruiert habe. Später wurde ein noch verbesserter angewandt, welcher in einer folgenden Figur verdeutlicht wird.

3. WIDERSTANDSDILLE.

(„Dim-a Lite“ von Wirt Co., Philadelphia, U.S.A.). Sie kontrolliert nur einfach die Intensität, stufenweise in vier Graden.

4. LAMPE.

„Mazda“ Tungstenlampe, 5, 10, 20, 50 u. 100 Kerzen.

„ Kohlenlampe, 1 Kerze u. a.

5. STRAHLENFILTER.



Textfigur 13.

i) Filterbassin (Fig. 13):—Ein Bassin mit parallelen Glaswänden, die ca. 1 cm weit voneinander entfernt sind.

ii) Farbenlösung:—Zu diesem Experimente gebrauchte Farbenlösungen sind: Rot, Orange, Grün, Blau, Gelb und Violett, unter denen nur die vier ersten ziemlich rein, monochromatisch,

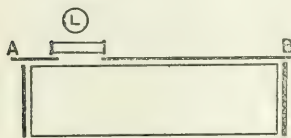
die letzten beiden klar polychromatisch sind.

Obschon sie unter einem Spektroskop examiniert und möglichst rein hergestellt wurden, so konnte ich doch aus Mangel an feineren Massstäben keine vollkommene Bestimmung ihrer Reihe im Spektrum erhalten. Je dünner, desto unreiner werden sie, also konzentriere ich die Farbe immer stärker, bis beinahe kein anderes Farbenlicht mehr durchgeht.

Farbe des Lichtes	Lösungsmittel	Dicke
Rot	Karmin u. Safranin	ca. 1 cm.
Orange	Orange G. Dr. Drüdlcr	„
Gelb	Kaliummonochromat	„
Grün	K-mono o. bichromat u. Methylenblau	„
Blau	Methylenblau	„
Violett	Gentianaviolett	„

III. Phototropismus.

1. BEI EBEN AUSGEBRÜTETEN GOLDFISCHEN.

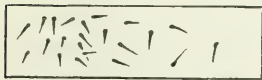


Textfigur 14.
Erklär im Text.

Die Fische waren nur 12—24 Stunden alt und wurden zu 50 in das Gefäß (Fig. 14) eingesetzt, von dem drei Wände mit schwarzer Pappe bedeckt waren, die vierte aber frei blieb, um die Bewegung der Fische zu sehen. Die Lichtquelle ist eine 5 k. Kohlenlampe, die Wasserhöhe ca. 9 cm. Die Strahlen fallen durch den Ausschnitt an

der Wand AB ein, der ca. 2/3 cm weit und ebenso hoch wie das Wasser ist.

FÜR WEISSES, ROTES UND GRÜNES :—Licht sind die Fischchen ganz deutlich positiv phototropisch, wie aus der nebenstehenden Skizze (Fig. 15) unzweifelhaft hervorgeht. Wenn der Karton (Fig. 14, AB) verschoben wird, so folgt die ganze Schar dem Lichte, bald nach rechts, bald nach links. Unter verschiedenen Farben-



Textfigur 15.
Erklär im Text.

lichtern finde ich keinen Unterschied, auch keine Abneigung gegen das Rot.

Nach einer Woche fand man keine Änderung in diesem Verhalten.

2. BIS EINEN MONAT ALTE LARBALENFISCHE.

(1—1,5 cm gross)

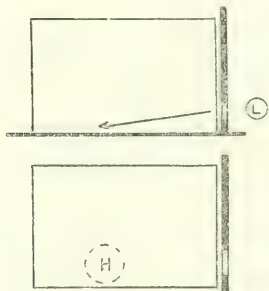
Einige von ihnen reagieren schwer, aber viele ebenso lebhaft positiv wie

die vorigen auf jedes Licht, weißes, rotes und grünes. Sobald die Lampe längs der Wand AB bewegt wird, schwimmen sie derselben munter nach. Auch hier konnte ich keine Rotschen beobachten

3. ZWEIJÄHRIGE GOLDFISCHE

(Grösse ca. 6—7 cm.)

10—15 Min. im Dunkel gehaltene Fische sind auch positiv phototrop für



Textfigur 16.
Erklär. im Text.

jedes Licht, weißes, rotes und grünes, wie die vorigen, aber nicht so lebhaft. Sie sammeln sich auf dem hellsten Platze (Fig. 16, H) bei jedem Licht. Photopische Fische wenden sich zunächst vom Licht ab, im Gegensatz zu vorigen, suchen aber auch allmählich den hellen Platz. Im ganzen sind ihre Bewegungen sehr ähnlich denen der folgenden dreijährigen Fische. Wie immer der Fall sein mag, konnte ich auch bei ihnen keinem Rotschen-Phänomen begegnen.

4. DREIJÄHRIGE GOLDFISCHE.

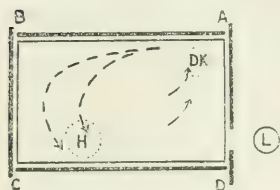
(Grösse ca. 10—12 cm.)

Für weisses Licht sind sie, falls skotopisch, auch positiv phototropisch, aber nicht so deutlich als die anderen; sie schwimmen umher, bald in, bald aus den Strahlen, aber versammeln sich nicht alle dauernd auf dem Ort (Fig. 16, II), besonders wenn der Strahl so stark ist, dass alle Teile im Aquarium ziemlich hell sind. Je dunkler es ist, desto leichter sammeln sie sich, und müssen wir ihre Bewegung bald nach der Beleuchtung sehen, sonst beginnen sie allgemein umherzuschwimmen. Jedoch konnte ich keine beobachten, die die Strahlen vermeiden oder das Dunkel suchen.

Wenn 5—10 Min. lang mit der 20 k. Lampe belichtet wird, halten sie sich zunächst an dunklen (Fig. 17, DK), der Lichtquelle entgegengesetzten Orten auf, schwimmen dann herum; sie sind für 2—3 Min. lieber negativ; dann werden sie allmählich positiv und kommen aus dem Dunkel hervor,

zuletzt auf (Textfig. 17, H.) Von dann schwimmen sie wie früher umher (vergl. v. Franz' Ansicht).

Bei diesem Experimente schliesse ich mein linkes Auge und sehe nur mit



Textfigur 17.
Erklärung im Text.

dem rechten die Bewegung der Fische. Deshalb, wenn das Licht ausgelöscht ist, ist mein rechtes Auge wie das der Fische photopisch, also kann ich mit dem rechten ebenso wie sie sehen, dazu noch mit dem linken deutlich ihre Bewegung beobachten. Sie sammeln sich erst in den dunkelsten Teilen (Textfig. 17, DK) und beginnen dann allmählich nach B zu schwimmen, darauf

nach H, zuletzt sammeln sie sich nach 2—3 Min. auf dem hellsten Platze. Je länger das Licht auf sie gerichtet war, desto später geschieht dies.

Für rotes Licht sind sie, wenn skotopisch, auch positiv; ich habe keinen Unterschied von vorigen Fällen bemerkt. Nur muss die Lampe wegen der stärkeren Lichtabsorption der Farbenlösung näher gebracht werden.

Photopische, mit 20 k. Lampe für 5—10 Min. belichtete, zeigen auch keine Änderung, sind auch positiv und beschreiben die Kurve ABC.

Für grünes u. gelbes Licht erweisen sich sowohl skotopische wie photopische auch positiv phototropisch; es zeigt sich kein Unterschied von vorigen.

Nach diesem Experiment sind die Goldfische (bis zu drei Jahren) für jedes (weisses, rotes und grünes) Licht positive Phototropen und jene Bauersche „Rotschen“, daß die Tiere, obwohl für das weiße Licht positiv, für das rote aber sehr scheu seien und es vermieden, wird nicht bemerkt. Umgekehrt sah ich sie manchmal nahe am roten Lichtfenster ruhig versammelt.

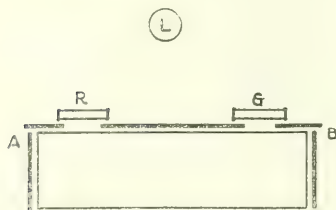
IV. Farbendiskrimination.

1. EBEN AUSGEBRÜTETE FISCHCHEN.

Gleichstellung zwischen rotem u. grünem Licht. Ein viereckiges Aquarium (Textfig. 18) gebraucht, das zwei Ausschnitte an der Wand AB hat, von denen einer rot (R), der andere grün (G) von einer Lichtquelle (L) belichtet wird.

Wenn das Licht in die Mitte gestellt wird, sammeln sie sich in Scharen

am grünen Ausschnitte, wo dann der hellste Teil des Gefässes ist. Wird die



Textfigur 18.
Erklär. im Text. IV. 1.

Lampe mehr rechts geschoben, nach dem roten Schmitte, so dass jetzt dieser heller beleuchtet ist, so sammeln sie sich jetzt an dem roten Ausschnitte. Nun schiebe ich die Lampe wieder links, nach der vorigen Stellung, bis die Lichtintensität der beiden Teile beinahe gleich aussieht; dann sammeln sich beinahe gleiche Scharen von ihnen

an den beiden Ausschnitten. Wir können ihre Bewegung beliebig mit der Lampe beherrschen. Diese Tatsache beweist uns, dass ihre Gleichstellung oder tropische Reaktion nur von der Lichtintensität und keinem Farbenton abhängt, und weder Abneigung noch Vorliebe vorhanden ist; also auch hier konnte ich keinem Bauerschen Phänomen entgegen.

2. EINEN MONAT ALTE FISCHE.

Zwischen Weiss und Rot, Weiss und Grün, Rot und Grün wird kein Unterscheid beobachtet, und keine spezielle Abneigung oder Vorliebe gegen oder für die Farbe erkannt, wie bei den vorigen Versuchen.

3. DREIJÄHRIGE FISCHE.

In diesem Falle ist es auch ganz so wie vorher. Nachdem ich sie im Rot sammeln liess, wurde die Lampe im Photokasten weiter geschoben, bis jetzt das andere viel heller mit blossen Augen gesehen wurde, dann schwammen sie nach dieser stärkeren Farbe. Wird das Rot wieder stärker, oder die andere Farbe schwächer gemacht, das rote Licht also heller als das andere aussieht, dann kehren die Fische wieder ins rote Licht zurück. Ganz ähnlich zwischen Rot und Orange, Rot und Grün, Rot und Blau. Die Auswahl fand nur nach der Intensität, nicht nach den Farbenton statt.

Nach dieser Beobachtung sind alle bis drei Jahre alten Goldfische im allgemeinen positive Phototropen, natürlich respondieren einige von ihnen nicht so klar wie andere, doch ist leicht zu vermuten, dass solche Phänomene von

verschiedenen Faktoren beeinflusst einige Ausnahmen zeigen. Die eben ausgebrüteten Fischchen sind bei der Wanderung am lebhaftesten und je älter, desto träger scheinen sie zu werden.

Hinsichtlich der Diskrimination unter verschiedenen Lichten giebt es bei ihnen weder Vorliebe noch Abneigung für oder gegen spezielle Farben, sodass ihre Wanderung nur von der Intensität des Lichtes abhängt.

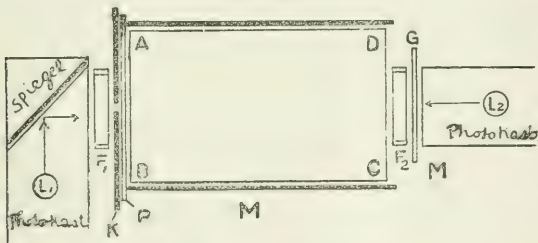
Also, ich kann kein Bauersches „Rotscheu“ Phänomen bei ihnen beobachten, sondern die Fische sind positiv phototropisch für alle Farben, darum können wir in diesem Gebiet keine spezielle Stütze für den Farbensinn der Fische finden.

V. Vergleichung.

Eine Methode den Farbensinn bei Fischen zu bestimmen.

1. VORRICHTUNG. (Textfig. 19)

Ein Aquarium, dessen zwei Längswände durch schwarze Kartons vor seitlich einfallendem Licht geschützt sind. Dicht an der Wand AB wird ein



Textfigur 19. Erklärt im Text.

dünnes, weißes Papier (P) angebracht, an dem ein Karton (K) aussen sich befindet, aus welchem an passender Stelle (im Mittel 2–3 cm hoch vom Boden) einige Punkte von der Form und Grösse des Futters ausgeschnitten sind. Vor dem K ist ein Photokasten in dem ein Spiegel 45° gegen die Achse des Kastens und die Wand des Aquariums steht. Wenn daher die Lampe (L₁) beleuchtet ist, werden einige Punkte von der Form und Grösse des Futters am weißen Papier beleuchtet. An der Wand CD steht ein anderer

Kasten mit einem matten Glas (G); die Lampe (L_1) in diesem Kasten kontrolliert (verändert) die Helligkeit an der entgegengesetzten weissen Papierfläche (P). Der Lichtfilter F_1 giebt die Futterfarbe; und der andere F_2 die Grundfarbe. Der Beobachter sitzt bei M und beobachtet, durch die Wasserschicht blickend, die Fische. Alle Versuche werden im Dunkelmzimmer vorgenommen, sodass in das Aquarium kein Licht außer dem von L_1 und durch den kleinen Ausschritt in (K) gelangen kann. L_1 ist eine 20 K. und L_2 eine 50 K. Tungstenlampe.

2. EXPERIMENT.

Goldfische, die schon ca. 1 Jahr lang mit rotem Futter aufgezogen worden sind, schnappen zu 80—90% nach ihrem gewöhnlichen roten Futter, wenn zugleich anders gefärbtes mit jenem gemischt gegeben wird.

1) Zuerst wird nur die weisse (weil in F_2 nur Leitungswasser sich befindet) Lampe L_2 erleuchtet. Ich lasse still das gewöhnliche rote Futter dicht vor der Wand AB (d. h. vor dem weissen Papier P) ins Aquarium fallen, welches bald von den Fischen gesehen und gefressen wird. Hierauf wird die Lampe L_1 erleuchtet. Wie oben schon berichtet, giebt das am Spiegel reflektierte Licht aus L_1 , gefärbt durch die rote Lösung im Lichtfilter F_1 , ein dem gewöhnlichen Futter im Form und Grösse beinahe gleiches Bild am weissen Kentischen Papier P. Einige Fische, vielleicht die ersten, die das Bild bemerkten, schwimmen herbei und schnappen nach der Wand. Nach einigen Versuchen schwimmen sie weg, nachdem sie das Futter als unerreichbar erkannt haben. Ihr Erinnerungsvermögen (dieser Unerreichbarkeit oder Nichtessbarkeit des Futters) ist ziemlich beträchlich und muss man dieses Experiment mindestens erst nach etwa zehn Minuten wiederholen.

2) Es scheint, also ob die Fische die Farbe sehr deutlich sehen. Entfernt man aber die Lampe L_1 vom Spiegel, während L_2 konstant bleibt, wobei die Helligkeit des roten Bildes geringer wird, obgleich man immer noch seine Röte deutlich sehen kann, so erkennen die Fische sie doch schon nicht mehr und schwimmen ohne Beachtung des Bildes längs der Wand vorbei.

3) Hierauf wird, während L_2 konstant bleibt, die Lampe L_1 näher an den Spiegel gebracht, dann tritt das rote Bild sehr deutlich an P auf, klarer als im vorigen Fall, die Röte kann man ganz deutlich am weissen hellen

Grund des Papier erkennen. Dennoch schwimmen die Fische achtlos längs der Wand vorbei, wie bei 2.

4) Lässt man nun die Lampe L_1 auf einer bestimmten Stelle, und nähert L_2 der Aquariumwand, so wird, das Aquarium photopisch (im Helligkeitsverhältnis zwischen Bild und Boden) wie bei 2, die Fische bemerken auch jetzt das Bild nicht.

5) Zuletzt wird L_2 von der Aquariumwand entfernt, was zur Folge hat, dass, wie bei 3, das rote Licht uns sehr deutlich erscheint, doch gehen die Fische achtlos längs der Wand vorbei.

3. SCHLUSS.

Wie gross immer die individuellen Unterschiede in ihrer Empfindlichkeit sein mögen, so konnten die Fische doch nicht die Futterfarbe von gewisser Helligkeit bei einer gewissen Bodenelligkeit erkennen. Besonders die Erscheinung, dass sie die Farbe nicht sehen konnten, wenn dieselbe uns klarer und deutlicher sichtbar wurde, beweist wohl den Mangel ihrer Empfindlichkeit für den Farbenton. Ihre entsprechende Bewegung nach der Farbe, die uns auf den ersten Blick als Farbenerkennbarkeit erscheinen möchte, bezieht sich augenscheinlich nicht auf die Farbe, sondern nur auf die Helligkeit des Gegenstandes. Kurz, die Fische scheinen mir farbenblind zu sein.

VI. Farbenwechsel bei Fischen.

1. FARBENSTOFFE DER FISCH.

Es gibt zwei Arten von Farbstoffen, schwarze Melanophoren und gelbe Xanthophoren, von denen die ersteren minder zahlreich und grösser, die letzteren zahlreicher aber kleiner sind. Ich kann keinen blauen Farbstoff bemerken; selbst bei den etwas bläulich aussehenden Teilen der schwarzen Karpfens erscheinen die inneren Melanophoren nur in durchfallendem Licht etwas blau, aber sie sind nichts anderes. Ausserdem gibt das Guanin in der Epidermis durch Reflexion, Refraktion u. a. den Körpern einen bläulichen Ton.

1. FARBENWECHSEL DER SCHWARZEN GOLDFISCHE

IM FARBENLICHTE.

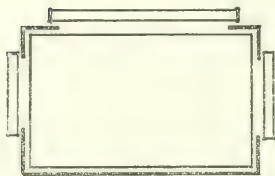
In der Doppelwanne (Fig. 23), deren Zwischenraum mit der Farblösung

(Saffranin-Rot und Gentiana-Violett) gefüllt wurde und die an südlichen Fenstern aufgestellt wurde, zeigen wochenlang darin lebende Fische verschiedene Expansion ihrer Melanophoren, die unter dem Mikroskop an ihren Schuppen sichtbar ist. Bei den in violetterm Licht lebenden Fischen (a) expandierten sie mehr als bei anderen, die im roten Licht (b) lebten (Taf. XLIV, Fig. 1.).

3. FARBENWECHSEL UNTER DAUERNDER REIZUNG VON VERSCHIEDENEN LICHTERN.

Das Aquarium (Textfig. 20) hat drei Fenster, eines an jeder Wand; alle anderen Teile sind mit schwarzer Pappe gedeckt.

Am 2. Nov. 1915 werden die Fische ins Aquarium eingesetzt und in jedem folgenden Monat untersucht. Sogleich nach dem Herausnehmen aus den beiden (roten und violetten) Aquarien, werden sie in kochendes Wasser getaucht, dann wird der Farbenton des ganzen Körpers mit blossen Augen und der Chromatophorenzustand unter dem Mikroskop vergleichend untersucht. Am



Textfigur 20.

22. Jan. aus den beiden Aquarien genommene Fische (Taf. XLIV, Fig. 2) hatten voneinander abweichende Farbtöne. Die im roten Lichte gewesenen (R) wurden rötlich-gelber, die im violetten Lichte (BD) bläulich-dunkel. Mikroskopisch konnte ich sehen, dass die schwarzen Melanophoren am meisten variierten, mehr als die gelben, wie in der Figur deutlich skizziert (Taf. XLIV, Fig. 3). Wie die Figur zeigt, vermehren die Melanophoren der im violetten Licht lebenden Fische (S.) sich entweder in Fläche oder in Menge, während die der im roten lebenden (R.) sehr kontrahieren, sodass selbst in dieser Figur der Ton der vorigen mehr dunkel aussieht. Aber mit dem Okularmikroskop gemessen, kam ich bei den gelben Chromatophoren keinen deutlichen Unterschied sehen, ob sie im roten Lichte in Fläche oder Menge zunehmen. In diesem Falle beruht daher der Farbenwechsel hauptsächlich auf der primären Melanophorenänderung, und die gelben Xanthophoren haben nur unterstützende, sekundäre Bedeutung, die Grundfarbe darzubieten.

Am 5. Feb. gekochte Fische zeigten das gleiche Resultat, aber weitere

eingehende Beobachtung in quantitativer Hinsicht ergab die Verhältnisse der Menge und Fläche der Xanthophoren und Melanophoren auf den beiden Schuppen. Die Fläche der gelben Xanthophoren ist beinahe ein Kreis, ca. $1,0-1,5\mu$ im Durchmesser, und beinahe gleich an den beiden Schuppen (nur die Farbenkonzentration verschieden). Dagegen war die Flächenbestimmung der Melanophoren unmöglich wegen ihrer expandierten Arme, und nicht so notwendig wie die vorige, weil die Figur genau ihre Unterschiede klar macht. Ich konnte auch eine Unterscheidung in ihrer Menge finden, wie folgend:

Durchschnittszahl der auf dorsalen Schuppen liegenden.

Farbenton	Melanophoren	Xanthophoren
der rotgelben Fische	46	16.5
der bläulichen Fische	91	9.5

Durchschnittszahl der auf allen Schuppen am Seitenorgan liegenden.

	Melanophoren	Xanthophoren
der rotgelben Fische	36	15
der bläulichen Fische	54	9

Wie diese Tabelle zeigt, vermehren sich auch die rotgelben Xanthophoren, aber es ist fast unmöglich ihre Menge einzeln zu zählen, so wird nur die Zahl in ca. $5 \times 3\mu^2$ auf den verschiedenen Flächen berechnet und bestimmt. Also die Zahl in der Tabelle ist relativ, während die der Melanophoren individuell gezählt worden ist. In Erwägung der letzteren muss natürlich die expandierte Fläche mit ihrer Zahlenzunahme in Betracht kommen. Ist die dauernde Belichtung mit violetter Farbe (dunkel) im Stande, die Melanophorenzahl zu vermehren und die Xanthophorenzahl zu vermindern? (BABACK und FRISCH). Wie erwähnt, werden die Fische im roten Licht gelbrot und im violetten bläulich dunkel, wegen der Vermehrung oder Verminderung, der Expansion oder Kontraktion ihrer Melanophoren und Xanthophoren. Dieser Wechsel war eine Tatsache, aber es ist eine andere Frage, wie er entstanden ist.

4. FARBENWECHSEL AUF FARBIGEM BODEN.

- 1) Acht Gefässe, jedes belegt mit einem farbigen Bodenpapier: Stark

und schwach Preussisch Blau (BS u. BD), stark und schwach Rot (RS u. RD), Weiss (W) und Schwarz (D_3), zwei graduierte Grau D_1 und D_2 . Der Boden jedes Gefässes ist beinahe von gleicher Grösse und auch jedes Papier, es ist also fast unnötig, um die Kleinheit des Gesichtswinkels besorgt zu sein, die v. Fischen in den HESS'schen Untersuchungen als seinen Irrtum verursacht zu haben angeführt wird. Dasselbe betrifft die Intensitätsdifferenz, die wegen der Grössendifferenz entstehen könnte. Alle Fische in jedem Gefäss werden auf weisses Papier versetzt und verglichen.

Die Fische, welche auf schwarzem Boden (D_3) gehalten wurden, sind viel schwärzer als die auf weissem (W), deren Melanophoren nicht so sehr expandieren wie die der vorigen (Taf. XLIV, Fig. 5,— E u. K). Dann wird ihr Grund gewechselt, die schwarzen werden auf weisses und die weissen auf schwarzes Papier gebracht; sobald ihre Vertauschung vollendet ist, beginnen die schwarzen Fische auf dem weissen Papier ihre Körperfärbung zu erholen, je in 30 Sekunden, längstens in 2 Minuten vollendet sich ihr Farbenwechsel. Ganz ähnlich auch die weissen Fische auf schwarzem Boden. Nach einiger Zeit werden beide ganz ähnlich aussehen. Die zu diesem Wechsel erforderliche Zeit ist für jeden Fisch mehr oder minder veränderlich, besonders die länger auf einem Boden



. Textfigur 21. Erklärung im Text.

gelebt haben, müssen länger auf dem anderen bleiben, um die Anpassungsfärbung zu gewinnen. In jedem Fall tritt der Wechsel zuerst an den dorsalen Seiten des Kör-

pers (Fig. 21, K), dann am Kopfe auf, dagegen bleibt die mittlere dorso-anteriore Linie (L) lange unverändert; dies bleibt oft nach vielen Tagen noch bemerkbar.

Zunächst werden die Körperfärbungen der auf jeden der verschiedenen Böden gelegten Fische beobachtet. Wie die eine Tabelle darstellt, gibt es drei Arten unter ihren Farbtönen: dunkel, rötlich gelb und bläulich dunkel, von denen die zweite Farbe so deutlich ist, dass sie niemand mit andern verwechseln kann, aber die letzte, die dunkelblaue, etwas zweifelhaft ist, weil wir keine anderen Farbstoffe finden konnten, die blau aussehen ausser den Melanophoren. Vielleicht lassen die optischen Verhältnisse der Melanophoren und besonders das Guanin sie so blau aussehen.

Wird nun der rote mit dem blauen Boden gewechselt, so dunkeln die früher rot aussehenden Fische, und die früher dunkeln gewinnen einen neuen roten Ton. Diese Änderung beginnt sogleich nach der Wechsellung, und vollendet sich in 30–120 Sekunden, und je länger die Zeit, desto deutlicher wird die Erscheinung. Auf weissen Boden versetzt, bleiben sie in derselben Zeit gleich.

Farbenton verglichen bei jedem Fische auf den verschiedenen
Farbenböden.

(a)

VI. 3. p.m. 1,30.—VI. 4. a.m. 9,30.

Boden- farbe Boden- farbe	BS	BD	RS	RD	W
BS					D. Blau.
BD	Dunk.				D. Blau.
RS	Dunk. Gelb rot.	Dunk. Gelb rot.			Gelb rot.
RD	Dunk. Gelb rot.	Dunk. Gelb rot.	Gelb rot. Dunk. Gelbr. Hell.		Hell Gelb rot.
D ₁			Dunk. (Gelb)	(Gelb) (Dunk)	Dunk.
D ₂	Dunk. (blau)	Dunk. (blau)	Gelb rot. Dunk.		Mehr Dunk.
D ₃	Dunk.	Dunk.			Meist Dunk.
D ₁				(β) Gelb?	
D ₂	(α) Hell (blau) Dunkel.				
D ₃			Hell Gelb. Dunk. (blau?)	(c) Hell Gelb. Dunkel.	

Wie ist diese Farbenänderung entstanden? Sind die Fische fähig, den Farbenton zu unterscheiden und sich ihm anzupassen? Wenn dies der Fall ist, können sie nicht farbenblind sein; sonst würden sie keinen Farbenton erkennen, sondern nur seine Lichtintensität. Eigentümlicherweise hängt dieser Wechsel von dem verschiedenen Expansionszustand ihrer Melanophoren und Xanthophoren ab, besonders der vorigen, und nicht vom Auftreten eines neuen Farbstoffes, selbst nicht bei den blau erscheinenden Fischen. Sehen wir die Tabelle an voriger Seite durch, dann vermögen wir zu erkennen, dass der Farbenton der RD Fische dem der D₁ Fische beinahe ähnlich aussieht. Was daraus vermutet wird, ist ihre (RD u. D₁) fast gleiche Reizungskraft für die Fische. Hat der Boden RD keine spezielle Farbenkraft und nur dem farblosen Grau D₁ entsprechende Reizungskraft? Sind die Fische dann monochromatisch, farbenblind?

2) Farbenwechsel geblendeter Fische. Bei der Blendung werden die Fische dunkler, wie die auf den schwarzen Boden gelegten, und zwar bald, ja während der Operation. Es ist sehr wahrscheinlich, dass die Blendung etwa gleiche Wirkung, nicht operatorische, auf die Fische wie das Dunkel hat, weil sie, wie die Menschen, nach der Blendung dunkel fühlen müssen. Diese geblendeten sind nicht mehr fähig sich der Umgebung anpassend zu verändern, und werden dann mehr und mehr schwarz. Aber die Blendung ist nicht einmal notwendig, es genügt schon, die Augen der Fische, z. B. mit den Händen, zu bedecken, um das Dunklerwerden der Hautfarbe zu veranlassen. Natürlich mögen bei diesem Fall die physiko-physiologischen wie psychischen Einflüsse während der Manipulation, z. B. Temperatur, Tastsinn u. a., eine Frage sein, aber der Wechsel der empfundenen Lichtintensität ist die hauptsächlichliche Ursache.

3) Farbenwechsel der abgezogenen Schuppen. Schwarzen, weissen, roten und blauen Fischen abgezogene dorsale Schuppen wurden im Wasser, in physiologischer Kochsalzlösung, Ringerscher Lösung und im Serum derselben Arten unmittelbar nach Abziehung unter dem Mikroskop beobachtet. In jedem Fall expandierten die Melanophoren zunächst in 2-4 Min. amöbisch (postmortale Expansion), dann begannen sie zu kontrahieren, und wurden umso schneller hell, je stärker die Belichtung war. Also sehen wir, dass die Abziehung wie jene Blendung gleiches Ergebnis bringt, und dies mag ein Beispiel sein,

welches beweist, dass Expansion und Kontraktion der Melanophoren von der Lichtintensität abhängen (Tonus). Taf. XLIV, Fig. 4. Melanophoren an Schuppen: E Expansion bald nach Abziehung und K Kontraktion nach der Belichtung.

Die Expansion und Kontraktion der gelben Xanthophoren wird ebenfalls bemerkt, aber nicht so deutlich wie die der Melanophoren, ausserdem verhalten sie sich diesen letzteren entgegengesetzt, indem sie im starken Licht expandieren und im dunkeln kontrahieren. In dem Fall der Melanophoren, welche amöbenartig sich bewegen, können wir selbst die schwarzen Melaninkörnchen in ihnen sich bewegen sehen, aber die Xanthophoren sehen nur öltropfenartig zerstreut aus und ihre granuläre Bewegung ist nicht wie bei vorigen sichtbar. Taf. XLIV, Fig. 5. Gelbe Xanthophoren an Schuppen: E nach Belichten und K vor Belichten.

Wie skizziert (Taf. XLIV, Fig. 4 u. 5) haben die expandierten Melanophoren (E) vielfach grössere Fläche als die kontrahierten (K) und bedecken die unter ihnen (d. h. innen) gelegenen gelben Chromatophoren, welche aber bei jener Kontraktion wiederum hervortreten, so dass wegen dieses neuen Auftretens und auch infolge ihrer Expansion der ganze Farbtom gelblicher wird. (Taf. XLIV, Fig. 7). Ausserdem wird wegen der Flächenverminderung der schwarzen Melanophoren (wegen der Vermehrung der weissen Fläche) das Aussehen heller (Taf. XLIV, Fig. 6). Daraus folgt also, dass die Farbenänderung der Schuppen, folglich der Versuchstiere, von der Expansion und Kontraktion der Melanophoren und Xanthophoren abhängt, welche von der Lichtintensität beeinflusst werden. Taf. XLIV, Fig. 4. Ein Melanophor bei Expansion und dasselbe bei Kontraktion. Fig. 6. u. 7. Änderung des Farbtoms vor und nach dem Belichten.

4) Farbenwechsel auf den Filtern, seine Intensität beliebig kontrollierbar. Wie schon gesagt, scheinen die Karpfen und Goldfische ihre Körperfarbe dem farbigen Boden anpassend zu wechseln, aber die auf den dünn roten und dünn grauen Boden gelegten Fische sehen untereinander beinahe gleich aus; darum fragte ich mich, ob vielleicht das Rot einen dem Grau entsprechenden und keinen eigenen Reizwert habe. Auch wurde die Farbe der Schuppe von der Lichtintensität verändert. Wird die Farbe also nur durch die Intensität bestimmt? Sind die Fische mangelhaft in der Farbeempfindung? Ändern sie sich nicht nach dem farbigen Boden, sondern der

Lichtintensität sich anpassen? Sind sie in Wahrheit farbenblind? Diese Aufgabe muss also noch weiter untersucht werden.

Vorrichtung. In diesem Fall steht der Photokasten senkrecht, auf den eine Mattglasscheibe (G) mit zwei, ca. 0,5 cm hohen Füßen (F) gelegt wird.

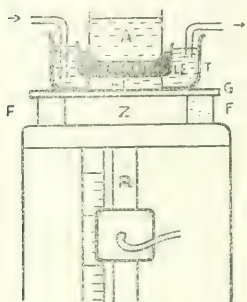


Fig. 22.

Die heiße Luft entweicht durch den oberen Zwischenraum (Z) und die Seitenrinne (R). Auf die Mattglasplatte (G) wird das Glasgefäß (T) gestellt, dessen innerster Boden eine kleinere Fläche als das Glas hat, um die Kleinheit des Gesichtswinkels zu vermeiden. Das innerste Gefäß (Aquarium A) wird ins äussere (Lichtfilter LF) auf Glasfüsse gestellt. Dann färbt das durch den Lichtfilter strahlende chromatische Licht den ganzen Boden des innersten Gefässes (A), und die Intensität kann beliebig mittels der beweglichen Lampe (L)

und jenes Widerstandes („Dim-a-lite“) kontrolliert werden. Die Temperatur des Fischwassers im Aquarium konstant zu halten, wird die äusserste Wanne benutzt, in die Wasser mit konstanter Temperatur geleitet wird. Als Filter wird Saffrano-Karmin- und Methylenblauwasserlösung gebraucht.

Beobachtung. Von Fischen, die auf weissem Boden gehalten worden waren, werden zwei ganz gleiche Fische ausgewählt, und einer ins rote, der andere ins blaue Aquarium gesetzt. Wie auf dem Papierboden, beginnen sie bald ihre Farbe zu ändern, und werden in ca. 5 Min. deutlich, die auf dem roten gelb, die auf dem blauen dunkel. Dann erniedrigt man die Intensität des Rot und verstärkt die des Blau so sehr, dass die stärkere Intensität des Blau ganz deutlich erscheint. Zu unserem Erstaunen werden die auf dem roten Boden schwimmenden Fische dunkel, die noch vor mehreren Minuten hellgelb waren, und die auf dem blauen werden nun hellgelb, während sie vorher dunkel waren. Obgleich dann die Intensität niedriger ist, so sieht dieses Rot noch ganz rot für unser Auge aus. Wenn auch die Intensität des Rotes vermehrt wird, so sehen die Fische dunkler als die auf dem blauen Boden schwimmenden aus, solange die Intensität des Blauen stärker ist, trotzdem das Rot ziemlich hell erscheint: Der Farbenwechsel

dieser Fische ist also beliebig von der Lichtintensität kontrollierbar, und hängt nicht von der Lichtfarbe ab, also sind demnach die Fische vielleicht, von diesem Standpunkte aus, farbenblind-ähnlich.

VII. Zusammenfassung.

1. Die Goldfische und die Karpfen zeigen positiven Phototropismus, aber ältere nicht so deutlich wie junge.
2. Lichtreiz kontrahiert die schwarzen Melanophoren und expandiert die gelben Xanthophoren, welche auf den Fischkörpern sich befinden.
3. Ihre Diskrimination zwischen den verschiedenen Farbenlichtern hängt nur von der Lichtintensität, nicht von dem Farbenton ab, und das BAUER'sche „Rotscheu“ Phänomen tritt bei diesen Fischen nicht auf.
4. In Vergleichung können sie das Rot von einem Grau nicht unterscheiden, wenn auch ich das Rot farbig zu erkennen vermochte.
5. Ihr Anpassungsfarbenwechsel an den Boden beruht auf der Lichtintensität, nicht auf dem Farbenton.
6. Daher schliesse ich vorläufig auf einen Mangel der Farbenempfindung bei diesen Fischen.

Anhang.

Farbenwechsel der Krustazeen Dekapoden (*Etyephra* sp.)

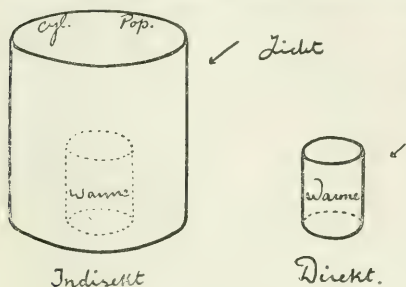
1. ETYEPHERA sp.

Viele von diesen leben im Teiche zu Komaba, wo die Wassertiefe nur 15–25 cm ist. Ihre Grösse ist meistens 1,5–3,5 cm. Nachdem sie gefangen worden sind, werden sie für 1–2 Tage im Glasgefäß ruhig gelassen, damit sie sich an die neuen Bedingungen gewöhnen, und dann erst zu diesem Versuche gebraucht.

2. FARBSTOFF UND FARBENTON.

Was man unter dem Mikroskop sehen kann, ist nur der Farbstoff, der ganze Farbenton dagegen kann nur mit blossen Augen beobachtet werden. Unter diesen Farbstoffen findet man drei Hauptarten: Blau, Gelb und Braun (Fig. 8); ausserdem selten Zinnoberrot (Taf. XLIV, Fig. 8, 2) und Grün

Farbenlicht aus, das sonst in die innere Wanne einfallen kann. Diese Doppelwanne wird nun auf weisses Papier (WP) und Glasschale (Sch) gelegt. Eine Glasflasche (C), in der die Tiere sich befinden, wird in die innere Wanne eingesetzt, so dass farbiges Licht auf sie fallen soll. Ich stelle diese Wanne an einem südlichen Fenster mit Mattglasscheiben auf, von denen zwei im direkten, hellem Licht und andere zwei, mit dickem zylindrischen Papier umgeben, im indirekten, dunkeln Licht stehen, um die Lichtintensität unter



Textfigur 24.

gleichen Verhältnissen einfach zu kontrollieren, wie in Textfigur 24. Daher hatte ich vier Wannen: hellblaue und hellrote, dunkelblaue und dunkelrote. Andere zwei Gruppen von Kontrolltieren, von denen eine in einem hellen und die andere in einem mit schwarzer Pappe umgebenen, ganz dunkeln Gefäss gehalten wird,

stelle ich auch mit den anderen farbigen Wannen in derselben Linie am gleichen Fenster auf. Diese vor dem Experiment in ihren Farbentönen ganz gleich aussehenden Tiere werden täglich, alle 24 Stunden einmal, in ihren Gefässen auf weissem Papier bei auffallendem Licht beobachtet.

4. FARBENLICHT UND FARBENWECHSEL.

Die Tiere in der Glaswanne auf weissem Papier werden allmählich farbenlos und durchsichtig wie das Wasser und das Glas, und ihre sehr kontrahierten Farbstoffe sind nur noch spärlich vorhanden. Diese beinahe farbenlosen Tiere ändern in jedem (Blau oder Rot) Licht ihren Farbenton allmählich vom Blau zum Braun und in 1-2 Wochen erreichen sie das Maximum, Brenzbraun, das entweder die geblendeten oder in Finsternis kurz und in Dämmerung lang gelebt habenden Tiere ausnahmslos aufweisen. Ihre Braun-Änderung wird vielleicht von der Lichtintensität hervorgerufen, sonst müssten sie ihre speziellen, an jede Umgebung angepassten Farbentöne immer besitzen. Je dunkler ihre Wohnung war, desto schneller ändern sie die Farbe zu braun.

5. DEKAPITATION DER AUGEN UND FARBENWECHSEL.

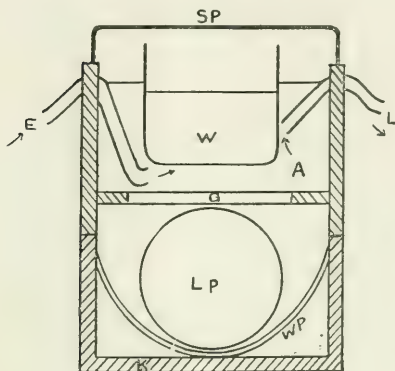
Wie oben erwähnt, werden die Tiere, denen beide Augen dekapitiert wurden, ausnahmslos braun und brennbraun. Dieses Phänomen beginnt prompt unmittelbar nach der Operation und wird meistens in 30–60 Minuten vollendet. Diese Änderung beruht hauptsächlich auf der Expansion der braunen Chromatophoren, während die Kontraktion der blauen nicht so deutlich ist, und lässt diese relative Vermehrung der braunen Fläche sie braun aussehen. Auch kann diese Braun-Änderung der geblendeten Tiere in jedem Licht ohne den mindesten Unterschied stattfinden. Es war auch interessant zu sehen, dass einige geblendete, in Blau-Licht gehaltene braune Tiere in weissem Licht dünn blau wurden, aber am folgenden Tage wurden sie wieder dünn braun, als ob sie schon unkontrollierbar oder achtlos der Wohnung geworden wären, Tiere, denen nur eines, das rechte oder linke, Auge extirpiert wurde, zeigen aber keine Braun-Änderung und auch keine seitliche Farbenunterscheidung im Körper, also die Lichtempfindung durch ein Auge erscheint ganz genügend zu sein, die Anpassung der beiden Körperseiten an die äussere Welt hervorzurufen. Aber eine weitere, strenge Beobachtung ergibt, dass diese auf einem Auge geblendeten Tiere etwas brauner als die zweiängigen Kontrolltiere geführt werden. Die Farbenänderung dieser Tiere zeigt auch, dass sie wenigstens von der durch ihre Augen empfundenen Lichtintensität beeinflusst werden. Wäre das nicht der Fall, so müssten sie, die geblendeten, ihre Körperfarbe ändern, sich der Umgebung anpassend wie die normalen. Ein abgeschnittener Teil des Körpers färbt sich auch braun (z. B. Abdomen und Telson).

6. LICHTREIZ.

Ein mit der Lichtwirkung verbundener Faktor, der bei diesen Experimenten nicht unberücksichtigt gelassen werden darf, ist die Wärmewirkung des Lichtes, welche z. B. die Krustaceen-Chromatophoren leicht ändern kann, so dass blaue Cyanokrustaceen ihre Farbe in rotes Krustacearubin ändern, und sind die Tiere sehr empfindlich für diese Hitzewirkung. Daher müssen beide Wirkungen von einander getrennt werden.

Eine die Tiere enthaltende Glaswanne (W) im Glasbodenaquarium (A) 1 cm über den Glasboden (G) gestützt und das neue Wasser unaufhörlich

durch den Einlass (E), das erwärmte durch den Auslass (L) geleitet; eine schwarze Pappe bedeckt das Aquarium oben. Das Ganze wird auf einen

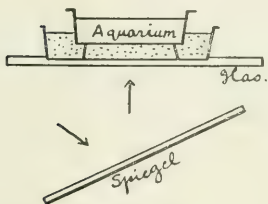


Textfigur 25. Erklär. im Text.

Photokasten (K) gestellt, in dem eine Lampe (LP) auf einem weissen Papier WP sich befindet. So erreicht das Licht, vom fließenden Wasser abgekühlt, durch den Glasboden die Tiere ventral. Die Kontrolltiere in der anderen Wanne werden während des Experiments neben dem Aquarium gehalten, ohne dass die Wirkung des Lampenlichtes auf sie gelangt, und beständig lässt man das gleiche Wasser um die Wanne so fließen,

dass die Temperatur in beiden Wannen gleich ist. Diese Wanne wird auf einem Mattglas mit Kammer-Licht belichtet.

Zu meiner Verwunderung wurden die Tiere in 50 Kerzen- (weisse und blaue etwas weniger Kerzenstärke) Licht eher etwas brauner, doch diese Änderung war nicht so deutlich und in einem Fall unmerkbar. Durchschnittliche Wassertemperatur 13°C, Belichtungszeit 5 Stunden. Im 100 Kerzen-Licht sehen die belichteten und die Kontrolltiere ganz gleich aus. Wirkt das direkte Kammerlicht stärker als dieses 50 K. Licht bei Belichtung für 5



Textfigur 26.

Stunden? Vermag also dieses 100 K. Licht beinahe gleiche Wirkungskraft zu haben? Und das weisse Solarlicht wirkt ganz gleich wie die Tungstenlampe, die etwas gelbes Licht gibt. Die direkte Strahlung der Mittagssonne im Juni hat eine Lichtwirkung von 100 K. Tungstenlampe. Blau- und Rotboden machen bei dieser Strahlung die Tiere auch gleicherweise braun. Eine andere

Tatsache wurde auch beobachtet, dass nämlich bisher in altem Leitungswasser gehaltene etwa 15 Braun-Tiere im direkten oder indirekten Solarlicht in 1-2 Minuten ganz blau und dann allmählich farbenlos wurden. Die Kontraktion und Expansion werden wie im anderen Fall sehr deutlich unter dem Mikroskop beobachtet.

7. SCHLUSS.

1. Drei Arten von Farbstoffen, Blau, Gelb und Braun, befinden sich in den Körpern der Tiere (*Etyophea* sp.) und die relative Menge dieser Farbstoffe spielt eine grosse Rolle in der Änderung ihrer Farbentöne.

2. Die Tiere werden im Kammer- und direkten Solarlicht zuletzt farbenlos und durchsichtig wie das Wasser oder das Glas.

3. Sie werden braun, in Finsternis vergleichsweise schnell, in Dämmerlicht ziemlich langsamer und in jedem (Blau oder Rot) farbigen Licht allmählich (Tonus bei der normalen Beleuchtung).

4. Dasselbe ist der Fall bei geblendeten Tieren und abgeschnittenen Teilen des Körpers; jedoch auf nur einem Auge geblendete Tiere zeigen die Erscheinung nicht so deutlich.

5. Diese Farbenänderung scheint letzten Endes nur von der Lichtmenge, der Intensität, abzuhängen; das Dunkel expandiert den Braunstoff (Krustaceorubin), kontrahiert den Blaustoff (Cyanokrustaceen), während Helligkeit ganz entgegengesetzte Wirkung ausübt.

An dieser Stelle möchte ich meinem hochverehrten Lehren, Herrn Prof. C. ISHIKAWA, meinen herzlichsten Dank aussprechen für das rege Interesse, welches er stets meiner Arbeit entgegengebracht hat.

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TAFELERKLÄRUNG.

TAFEL XLIV.

Fig. 1. Der Unterschied der Melanophorenexpansion der im violetten Licht lebenden (*a*, *c*) von der im roten Licht lebenden Tiere (*b*, *d*).

Fig. 2. Der Unterschied des Körpertons, *RG* dauernd im roten Licht gelebt, während *BD* im violetten.

Fig. 3. Der Chromatophorenzustand an der kaudalen Flosse des Fisches in Fig. 2. *S*₁ des *BD*, *R*₁ des *RG* Fisches unter schwacher Vergrößerung beobachtet; *S*₂ des *BD*, *R*₂ des *RG* Fisches noch stark vergrößert. *FM* bedeutet Flossenmembran, *FR*-Flossenstrahl.

Fig. 4. *E*, ein Melanophor bei Expansion; *K*, derselbe bei Kontraktion nach Beleuchtung.

Fig. 5. *K* und *E* sind dieselbe Xanthophorengruppe an Schuppe; vordere bei ihrer Kontraktion, letztere bei ihrer Expansion nach Beleuchtung.

Fig. 6. *E* und *K* sind das expandierte und kontrahierte Bild desselben Schuppenteils. Wir sehen hier zwei Arten der Melanophoren, die eine aussen gelagerte, sehr fein dikotomisch

geasterte, die andere innere, nicht so sehr geasterte Melanophoren, und sind diese grauer als jene.

Fig. 7. *E* und *K*. Beide Bilder desselben Schuppenteils; eines bei Expansion, das andere bei Kontraktion. Diese kontrahierten Melanophoren lassen die bisher bedeckten Xanthophoren auftreten, folglich wird das ganze Bild heller und mehr gelblich als *E*.

Diese vier (4—7) Figuren zeigen uns, wie der relative Farbenton und die Helligkeit der Schuppenfläche bei der Expansion und Kontraktion der Chromatophoren verändert werden kann.

Fig. 8. Chromatophoren von *Etyophera* sp. *B* die der Blautiere, *W* die der farblosen Tiere und *Br* die der Brauntiere, jede an Kalapasen. *I*, *II* u. *III* sind derselbe Körperteil, jedes Bild unterscheidet sich nur im Expansions- oder Kontraktionszustand der verschiedenen Chromatophoren. α u. α' , β u. β' sind dieselben. Bei *I* erscheinen die Tiere braun, bei *II* fast farblos (etwas gelblich oder grünlich) und bei *III* bläulich. An *a* und *b* sehen wir grünliche Farbstoffe, welche im durchfallenden Licht weiss (*c*) aussehen. *d* sind die Farbzellen bei farblosen Tieren. *z* seltsam auftretende Zinnoberfarbzelle.



Fig 7.

E.



K.

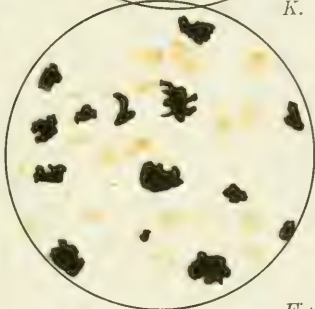


Fig 8.

α .

I.

β .

II.

α' .

W.

β .

III.

c.

b.

a.

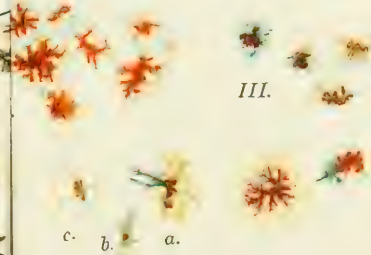


Fig. 1.

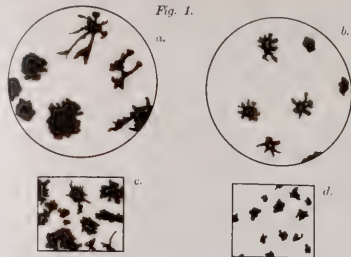


Fig. 4.



Fig. 7.

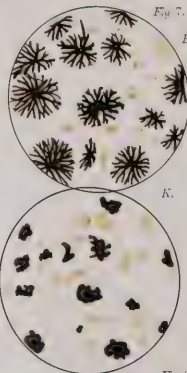


Fig. 2.

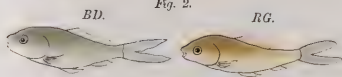


Fig. 5.

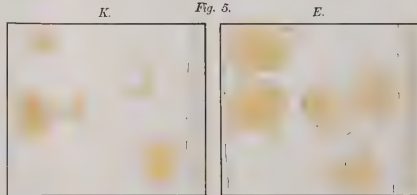


Fig. 3.

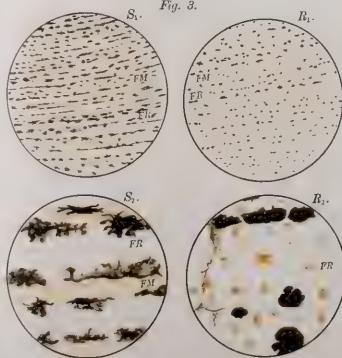


Fig. 8.

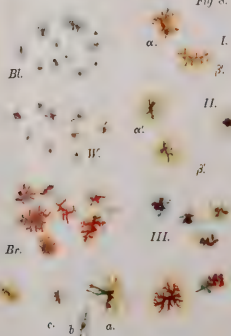
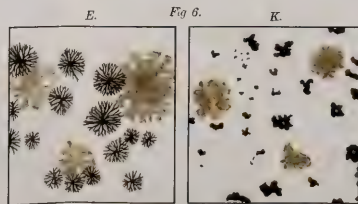


Fig. 6.



Einige Bemerkungen über den Farbenwechsel bei Karpfen durch physikalische Einflüsse.

VON

Yûnosuké Ohashi.

Aus dem Institut für Meeres-Zoologie.

Direktor: Prof. Dr. CHIYOMATSU ISHIKAWA.

Mit Tafel XLV und 7 Textfiguren.

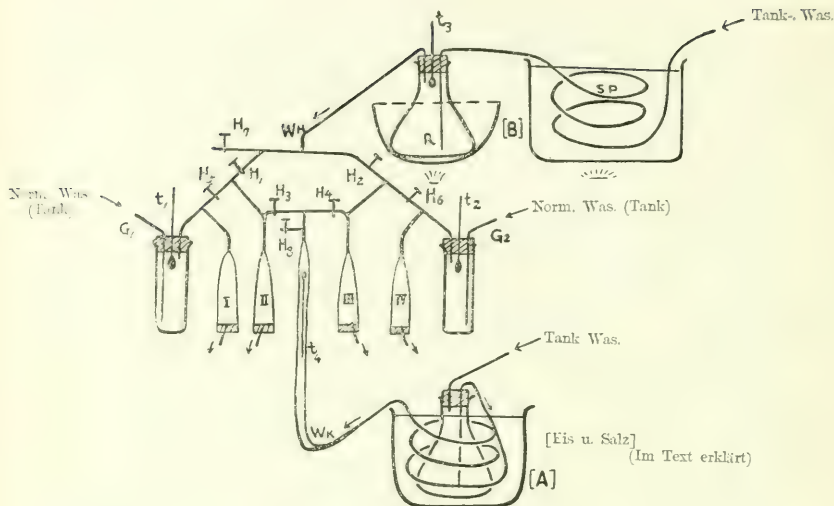
I. Wirkung der Temperatur auf die Farbe von lebenden Karpfen.

1. VERSUCHSANORDNUNG.

Es ist klar, dass die grössere oder geringere Menge eines im Wasser gelösten Gases die Temperatur dieses Wassers beeinflussen muss. Wenn man daher offene Gefässe braucht und in diesen die Temperatur erhöht wird, so muss der veränderte Gaszustand die Genauigkeit der Untersuchung beeinträchtigen (siehe IV). Wir bedürfen also eines geschlossenen Systems, um das Entweichen des Gases zu verhindern. Andererseits aber tritt im geschlossenen System wieder das Problem der Druckänderung infolge der Temperaturveränderung auf, das freilich vorläufig nicht berücksichtigt werden kann. Die untersuchten Karpfen sind sogenannte 3 jährige, 6-7 cm lange schwarze „Magoi.“

Das geschlossene System. Die Leitungsröhren sind, wie in Textfig. 1 dargestellt, eingerichtet. An den Enden jedes Rohres befindet sich ein Glasgefäss, in dem die Fische gehalten werden. I und IV dienen als Kontrolle, II und III sind für den Versuch bestimmt. Wenn nun H_1 , H_2 , H_3 , H_4 geschlossen und H_1 , H_2 * geöffnet sind, fliesst das normale Wasser durch die Glasgefässe G_1 und G_2 in die vier Röhren, dann kann man die Wassertemperatur an t_1 und t_2 ablesen.

* H heisst Hoffmann's Hahn.



Textfig. 1. Erklärung im Text.

Wasser von höherer als normaler Temperatur fließt immer durch den geöffneten Hahn H_7 ab, sonst nimmt die Wassertemperatur bald zu, d.h. das Wasser wird zu warm zum Versuche und man kann kein Wasser mit konstanter Temperatur beliebig lange Zeit in die Fischröhren senden. Wenn man hierauf wärmeren Wassers bedarf, schliesst man die Hähne H_1 , H_2 und H_7 , öffnet H_3 und H_4 , dann kann es in II und III geleitet werden. Die Temperatur des Wassers zeigt das Thermometer t_3 . Fig. 1 [B] ist ein Wasserbad, in dem ein spirales Rohr (sp) und Regulierungsgefäß (R) sich befinden, in dem ersten wird das Wasser erwärmt und in dem zweiten das erwärmte Wasser reguliert, so dass eine Temperaturschwankung nicht, wenigstens nicht plötzlich auftreten kann. t_3 ist das Thermometer.

Kaltes Wasser muss immer durch II abfließen, sonst gefriert das Wasser im spiralen Glasrohr nach einigen Minuten und letzteres zerspringt. Schliessung von H_1 , H_2 und H_7 , Öffnung von H_3 und H_4 gestatten das Einströmen des kalten Wassers in II und III. t_1 zeigt die Temperatur des Wassers an. In dem Abkühlungsapparat (Textfig. 1 [A]) befindet sich das Kühlmittel: Eis und

Salz (im Gewichtsverhältnis ca. 2:1). Das Wasser aus dem Tank wird erst in eine ERMENYERSche Flasche geleitet, wo es die erste Abkühlung erfährt, dann wird es durch das Spiralrohr noch mehr abgekühlt, geht in das Thermometerrohr (N), in dem die Temperatur gemessen wird, und zuletzt nach II und III.

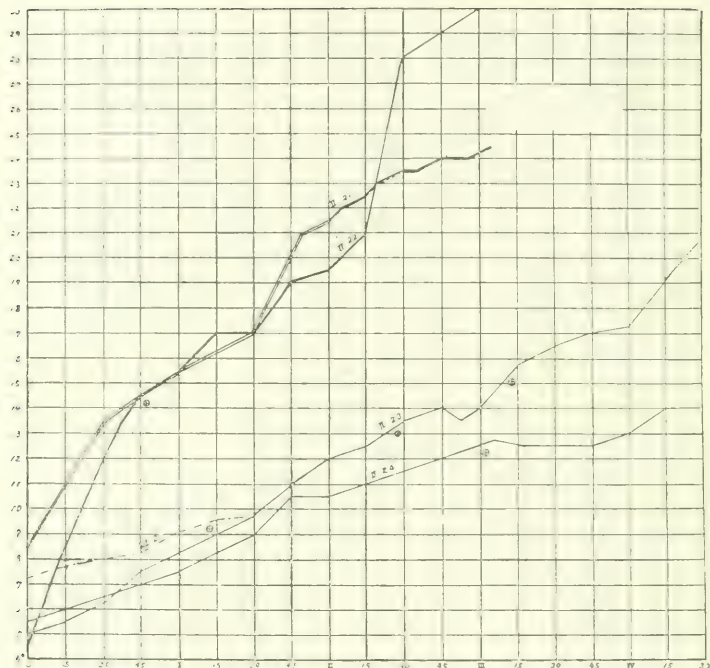
Diese vier Fischröhren stehen auf weissem Papier und die dem Boden sich anpassenden Karpfen werden sehr licht und hell, d. h. ihre Melanophoren kontrahieren. Zunächst werden vier ähnlich gefärbte Karpfen ausgewählt, welche alle, auf weissem Boden im Aquarium gefüttert, sehr hell geworden sind. Insbesondere jedes Paar I und II, III und IV muss sorgsam nicht nur auf ihre Schattierung, sondern auch auf ihren Farbenton verglichen und ausgewählt werden, sonst ist es schwer ihre Änderung zu beobachten.

2. TEMPERATUR-STEIGERUNG.

a) Plötzliche Erwärmung. Wenn die Hähne so geöffnet werden, dass warmes Wasser in die Gefässe II und III geleitet wird, werden die vorher auf Farbenwechsel kultivierten* Karpfen in etwa 1 Minute (besonders in der letzten halben Minute) deutlich dunkel. Ich glaube, dass das Dunkelwerden sobald nach dem Erwärmen auftritt, aber das in der Leitungsbahn noch bleibende normale Wasser erniedrigt in der ersten halben Minute die Wärme, und so zeigt sich die Dunkelung deutlicher erst in der zweiten halben Minute, wie unter der Lupe sehr klar beobachtet wird. Bei diesen plötzlichen Temperaturänderungen von 6.5° — 21° , 6.5° — 25° , 7° — 20° , 7° — 21° , 7° — 23° , 7° — 25° , 9° — 15° , 9° — 20° , 9° — 23° , 9° — 25° , 10° — 15° , 10° — 20° und 10° — 25° wird das Dunkelwerden der lebenden Karpfen ganz ähnlich beobachtet, grössere Temperaturerhöhung, z. B. bis zu 30° , giebt keine verschiedenen Resultate. Eben so leicht, in etwa 1 Minute, kann man auch die frühere Farbe wieder herstellen, wenn wieder normales Wasser hineingeleitet wird, und dieses Phänomen lässt sich mit gleichem Resultat beliebig wiederholen.

b) Allmähliche Erwärmung. Aber solche plötzliche Temperaturerhöhung tritt in der Natur kaum jemals auf, natürliche Temperaturänderungen finden

* Kultiviert heisst, die Fische wurden vor dem Versuche vielmal auf weissen und schwarzen Boden wechselnd gelegt, sodass ihr Farbenwechsel dann leicht auftrat; andere Karpfen zeigen die Veränderung natürlich langsamer.



Textfig. 2. Kurve I (ein Beispiel) Temperaturveränderung des Wassers bei allmählichen Erwärmungsversuchen inbezug auf die an der Körperfarbe des Karpfens. ⊙ zeigt die Zeit und Temperatur an, wo die Dunkelung sehr deutlich wurde.

in der Regel viel langsamer statt. So ist also der oben beschriebene Farbenwechsel bei Karpfen nur als ein abnormes Phänomen anzusehen, und es entsteht die Frage, ob wir dieses Dunkelwerden, wie v. Frisch sagte, auch bei allmählicher Temperaturerhöhung beobachten können. Die Temperatur wurde also allmählich, wie in Kurve I (Textfigur 2), gesteigert, eine vielleicht in der Natur mögliche Änderung, trotzdem geht die Dunkelung je in keiner langen Zeit vor; die allmähliche Ausbreitung der Melanophoren (hauptsächliche Ursache des Farbenänderung) kann man leicht unter einer niederen Lupe beobachten.

Die Marke \otimes in der Kurve zeigt die Zeit und Temperatur an, wo die Dunkelung sehr deutlich wurde.

Ob noch langsamer zunehmende Temperatur einen Effekt hat oder nicht, wurde noch nicht beobachtet, aber man kann wohl vermuten, dass das Phänomen auch hier nach einer gewissen Zeit auftritt.

3. TEMPERATUR-ERNIEDRIGUNG.

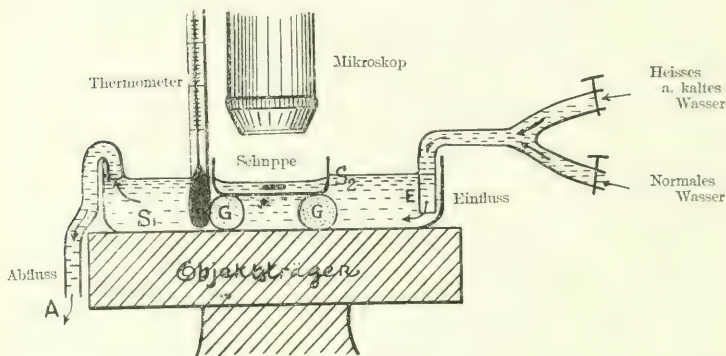
Zu meiner Verwunderung wurde auch hierbei ein Dunkelwerden der Körperfarbe beobachtet, und vielmalige Wiederholung des Versuchs liess keinen Zweifel an der Wirklichkeit des Phänomens. Erniedrigung von $10^{\circ}-5^{\circ}$, $10^{\circ}-4,5^{\circ}$, $9^{\circ}-5^{\circ}$ und $9^{\circ}-4,5^{\circ}$ wirken ganz ähnlich, auch hier werden die auf Farbenwechsel kultivierten Karpfen in 1 Minute ganz dunkel, besonders in der letzten halben, wie bei Erwärmung. Allmähliche Änderung wurde noch nicht untersucht, aber man kann wohl auch dann die gleiche Erscheinung vermuten,

4. ANPASSUNG DER KARPFFEN IM WARMEN WASSER.

Aus dem oben beschriebenen Farbenwechsel kann man zunächst annehmen, dass, wenn die Karpfen immer im warmen Wasser leben, sie sich erstens der Wärme anpassen (MAYERHOFER'sche Hechte), und zweitens wie im normalen Wasser hell oder dunkel sich dem weissen oder schwarzen Boden anpassen. So wurden die Gefässe I und II auf weissen, III und IV auf schwarzen Boden gelegt, normales Wasser in I und IV, warmes in II und III geleitet, dann wurden II mit I, III mit IV verglichen. Aber im warmen Wasser können sie sich dem weissen Boden im Laufe von mindestens 1 Stunde nicht anpassen, während die Einführung normalen Wassers die Anpassung plötzlich, in 1 Minute, hervorbringt. Die bisher 30 Minuten im wärmeren Wasser auf schwarzem Boden gelagerten Fische werden nun auf weissen übergesetzt, aber auch diese können in 1 Stunde sich nicht anpassen, während die in normalem Wasser auf schwarzen Boden IV in 1 Minute denen in I, die vom Beginn immer im normalen Wasser auf weissen Grund gelegen haben, ganz ähnlich aussehen werden. Also sehen wir, dass eine Stunde für Karpfen nicht genug ist, die Wirkung der Temperaturänderung null zu machen, nämlich sie der Wärme und dem weissen Boden gung anzupassen.

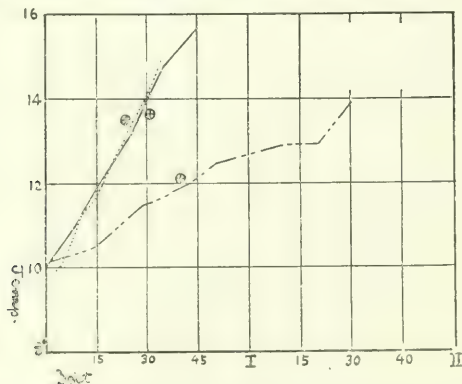
II. Temperaturwirkung auf die Schuppen-Melanophoren.

1. ANORDNUNG.



Textfig. 3. Erklärung im Text.

Wie Fig. 3, wird das beliebig erwärmte Wasser durch E in die äussere Schale S_1 geleitet, dann fliesst es durch die Röhre A aus, also die Temperatur der Flüssigkeit in Schale S_2 wird immer beliebig kontrolliert. Die Temperatur



Textfig. 4. Kurve II (ein Beispiel). Kurve der bzw. allmählichen Temperatursteigerung der Flüssigkeit, in der abgezogene Schuppen d. h. zu beobachtende Melanophoren, sich befinden.

kann man am Thermometer lesen. Abgelegte Schuppen beobachtet man in physiologischer Kochsalzlösung, Ringer'scher Lösung oder Serum derselben Tiere unter dem Mikroskop.

2. TEMPERATURSTEIGERUNG.

Die Melanophoren beginnen bald nach dem Abschuppen sich allmählich auszubreiten, was etwa 30 Minuten dauert, während das Tankwasser (9° — 10°C) immer in S_1 fließt. Wird dann warmes, vorher zu lebenden Karpfen geleitetes Wasser (20° — 25°C) in S_1 eingelassen, so beginnen die Melanophoren sich zu kontrahieren, aber ich konnte keine vollständige Kontraktion beobachten (sie bleiben im mittleren Zustand), als ob die plötzliche Temperaturänderung ihre Reaktionsfähigkeit herabgesetzt oder narkotisiert habe. Immerhin kann man im ersten Augenblick ihre Kontraktion bei höherer Temperatur leicht beobachten.

Wird hierauf, wie z. B. in Kurve II (Textfig. 4) gezeigt, die Temperatur verhältnismässig allmählich erhöht, so erscheint nun die Melanophorenkontraktion glätter und allmählich wie in Taf. XLV, A, 1 u. 2; B, 6 u. 7, zuletzt ganz vollständig (ibid. A, 3), was man vorher nicht beobachten konnte. Nach der vollständigen Kontraktion wird die Temperatur wieder erniedrigt, dann breiten sich die Melanophoren nach und nach wieder aus, und kehren zuletzt zu ihrem vorigen Zustand zurück (Taf. XLV, A, 4 u. 5; B, 3). Aber diese ausgebreiteten können nicht wieder ganz kontrahiert werden, wie oben schon erwähnt, wenn auch die Temperatur, in der sie bisher vollständig kontrahierten, plötzlich wieder hergestellt wird. Nur einmal, als die Temperatur noch langsamer erhöht wurde, kontrahierten die Melanophoren nicht nur in der niederen, sondern auch in der höheren Temperatur. Entweder war diese Temperaturänderung zu mild, um ihre Kontraktion zu erregen, oder sie hatten unterdes ihre Reaktionsfähigkeit verloren, gleichsam, als ob sie narkotisiert worden wären.

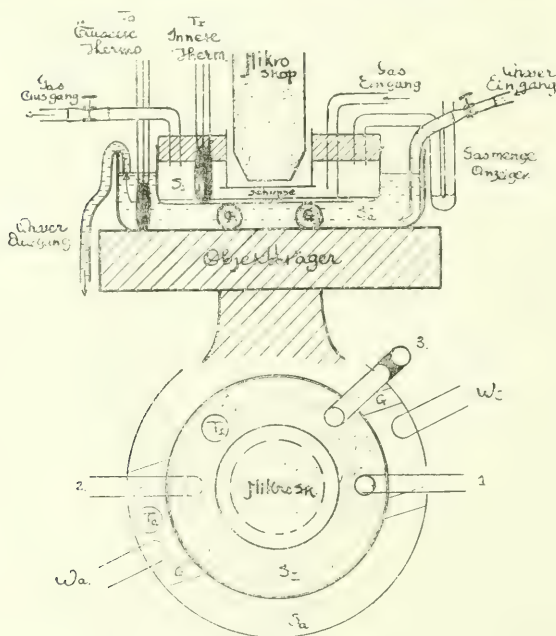
III. Wirkung von Gasen auf die Farbe lebender Karpfen.

A. WIRKUNG VON SAUERSTOFF.

1) Versuchsanordnung. Das Wasser, einmal in den Tank geleitet, fließt aus diesem wieder in den Gaskühler GK ein, und gelangt durch das Rohr R_1

stündigen (mindestens 5-6) Einströmen gemacht werden sollte. Doch ist die Wirkung nicht so deutlich wie in den vorigen Fällen, obgleich die Fische sich tatsächlich auch in 1-2 Minuten etwas dunkel veränderten, aber nach Einfließen armer oder reicher Gaslösung passen sie sich dem weissen Boden je in der dritten Minute an oder bleiben einige schwarz (unangepasst) bis zu 1,5 Stunde.

Diese Unklarheit der Veränderung beruht nicht nur auf der gelösten Gasmenge, sondern auch und hauptsächlich auf dem Charakter dieses Gases (Wasserstoff ist wirkungslos auf den Lebenden). Man kann vermuten, dass das Gas zunächst auf den Kreislauf der lebenden Fische wirkt, und dann direkt oder indirekt den Farbenwechsel hervorbringt. Also ist als wahrscheinlich zu vermuten, dass die bei der Atmung die wichtigste Rolle spielenden



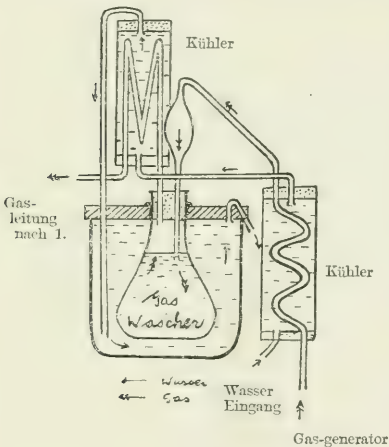
Textfig. 6. Erklärung im Text.

Gase O_2 und CO_2 die Farbenänderung am deutlichsten auftreten lassen. Vielleicht beruht die dabei auftretende leichte Veränderung auf dem Sauerstoffanangel, der durch das Einblasen des Wasserstoffs hervorgerufen wird.

IV. Gaswirkung auf die lebenden Melanophoren der Schuppen.

1. VERSUCHSMETHODE.

Fig. 6. Von den zwei Schalen auf dem Objektträger dient die äussere S_2 , in der immer konstant temperiertes Wasser fliesst, die innere S_1 immer auf



Textfig. 7. Erklärung im Text.

konstanter Temperatur zu halten; das Thermometer T_2 zeigt ihre Wassertemperatur. Die Schale S_1 hat eine Korkdecke mit einem Thermometer T_1 und vier Glasröhren, von denen in die mittlere, grösste die Objektlinse eingetrieben wird, so dass man sehr vergrösserte Pigmente sehen kann; bei Wassertropfen in Röhre 3 erweist, wie viel Gasmenge in S_1 ein- und ausgeht. Das Thermometer T_1 zeigt die Temperatur der inneren Schale. Dem im Generator entwickelten Gas, das zuerst durch Gaswaschen (Fig. 7) von Säurespuren und andern frei geworden

ist, wird durch das Glasrohr im Kühler die Entwicklungswärme genommen, dann wird es durch das Rohr 1 (Fig. 6) eingesandt und durch 2 ausgehen gelassen. Die Schuppen befinden sich auf dem Boden der Schale S_1 , lebend in der physiologischen Kochsalzlösung.

2. RESULTAT.

(Wirkung von Sauerstoff, Kohlensäure und Wasserstoff)

Auch hier zeigen alle drei Gase dieselbe Wirkung; die expandierten

postmortale und auch auf der NaCl-Wirkung beruhende Veränderung Melanophoren der Schuppen beginnen ihre Kontraktion, sobald jedes Gas in S. gesandt wird. Aus mehreren Versuchsnoten führe ich folgendes an:

1917, VI, 23.

9. 2 a.m. Abschuppen u. die Schuppen in die Lösung.
Temperatur $18,75^{\circ}\text{C}$ (T_a)
Allmählich expandieren die Melanophoren.
- 9.53 am weitesten exp.
- do. (+CO₂) $18,6^{\circ}\text{C}$ (T_a). $19,7^{\circ}\text{C}$ (T_1).
bald eintretende Kontraktion.
10. 5 (-CO₂) halbe Kontraktion.
- 10.45 Ganz Kont., das zuerst kontrahierende Pigment begann die zweite Exp.
- 10.15 p.m. Fast ganze Exp., aber einige noch nicht so sehr exp.
-
- 10.16,5 (+O₂) $19,75^{\circ}\text{C}$ (T_a) $20,35^{\circ}\text{C}$ (T_1)
Kont. tritt bald ein und schreitet allmählich fort.
- 10.25 (-O₂) halbe Kont. $20,5^{\circ}\text{C}$ (T_1)
Melanophoren (zentrale, G) halbe Kont., viele periphere (P) expand. noch. Mit der Zeit kont. beide Arten.
- 0.42 Ganze Kont. und zweite Exp.
- 1.30 Ganze Expansion.
-
- 1.32,5 (+H) $19,75^{\circ}\text{C}$ (T_a) $20,4^{\circ}\text{C}$ (T_1)
Kont. tritt bald ein.
- 1.53 Melanophoren (G) beinahe ganz kontrahiert, während P eben ihre Kont. begannen.
- do. (-H)
- 2.00 Einige expandiert.
- 3.00 die meisten expandiert.

IV. Elektrische Wirkung auf die Farbe der lebenden Karpfen.

Konstanter oder Induktionsstrom wird in die Fischenröhre Fig. 1 geleitet; die Schliessung des Stromes verdunkelt bald, dann mehr und mehr zunehmend

die Farbe der Karpfen. Aber bald nach der Öffnung tritt wieder allmähliche Anpassung an den weissen Boden ein, die man, solange der Reiz dauert, nicht beobachten kann. Diese Dunkelung vollzieht sich in 30–60 Minuten. Es gibt keinen Unterschied zwischen dem konstanten- und Induktionsstrom. Allmähliche elektrische Wirkung wurde noch nicht versucht.

V. Elektrische Wirkung auf die lebenden Melanophoren der Schuppen.

Beide Pole des konstanten- oder des Induktionsstroms in der inneren Schale S; Fig. 2, in der die Schuppe sich befindet. Bei der Schliessung kontrahieren die hierzu gut expandierten Melanophoren sehr schnell und die Öffnung lässt sie wieder, wie vorher, expandieren, aber dieses Phänomen findet ganz schnell statt, und wenn der Strom länger als eine Sekunde einwirkt, so scheinen die Melanophoren schon ihre Reaktionskraft zu verlieren. Also muss man den Schlüssel sofort öffnen, sobald die Kontraktion auftritt, um wieder die Expansion beobachten zu können. Folgendes als Beispiel:

1917, III, 26.

- | | | | |
|---------------------------------|------|--|--------------------------|
| 11 ^h 16 ^m | a.m. | Abschuppen. | 12,8°C (S ₁) |
| 12. 17 | | Grösste Exp. | 10,1° |
| 12. 20 | | Schliessen des Stromes. | |
| 12. 20,5 | | (augenblicklich) Kontraktion deutlich (10,1°C), aber periphere Melanophoren noch nicht so deutlich kontrahiert, als die zentralen. | |
| 12. 20,6 | | Öffnung des Stromes,
bald Expansion, (10,1°C). | |

Diese Kontraktion der Melanophoren kann man noch deutlicher an den Schuppen auf der Platinelektrode des Induktionsstroms beobachten.

VI. Ergebnisse.

1. Lebende Karpfen ändern sich dunkel, d. h. lebende Melanophoren der Schuppen der lebenden Karpfen expandieren infolge von plötzlicher wie allmählicher Erwärmung oder Abkühlung.

2. Im an Sauerstoff oder Kohlensäure reichem Wasser, ferner in dem mit Wasserstoff beladenen oder gekochten, d. h. weniger (als normales) Gas enthaltenen Wasser, und auch

3. bei der elektischen Wirkung des konstanten wie des Induktionsstromes, sowie.

4. bei jeder äusseren Störung oder Reizung scheint die Dunkelung des lebenden Fischkörpers aufzutreten. Also z. B. wenn die Schnelligkeit des Wasserstroms in den Fischröhren (Fig. 1) verändert wird, kann man auch ihre Dunkelung beobachten, selbst wenn die Temperatur des Wassers in beiden, Versuchs- und Kontrollröhre, gleich geblieben ist.

5. Und die Tatsache dass die Melanophoren des Fischenkörpers nach dem Tode sich erweitern, dass sie an abgezogenen Schuppen nach der Operation noch immer mehr expandieren, dass auch bei Fischen, denen Atropin injiziert worden ist, ihre Expansion beobachtet wird- alles dies beweist, dass die Melanophoren einen peripheren Tonus besitzen müssen, der zusammenziehend auf sie wirkt. (Diese Atropinwirkung soll noch weiter studiert werden.)

6. Dagegen kontrahieren die lebenden Melanophoren an abgezogenen Schuppen in der physiologischen Kochsalzlösung, Ringer'schen Lösung und in Karpfenserum durch die gleichen oben beschriebenen Einflüsse. In diesem Falle wirken also die gleichen Ursachen in der entgegengesetzten Weise. Ob diese Kontraktion durch direkte Wirkung auf die Melanophoren oder indirekt durch den Einfluss auf ihre Nervenendigungen hervorgerufen wird, muss noch weiter untersucht werden.

Zum Schluss meiner Arbeit ist es mir eine angenehme Pflicht, meinem hochverehrten Lehrer Herrn Prof. Dr. C. ISHIKAWA nicht nur für die Anregung zu dieser Arbeit, sondern für sein mir immer zuteil wurde, meinen Dank auszusprechen.

TAFELERKLÄRUNG.

TAFEL XLV.

Fig. 1-5. Eine Gruppe von Melanophoren an abgezogenen Karpfenschuppen, die Kontraktionszustände in verschiedenen Temperaturen und Zeiten zeigend.

Fig. 1. Vor der Temperatur einwirkung.

Fig. 2. 9.50—9.55 a.m. 12.4°—13.3°C.

Fig. 3. 10.20 a.m. 18.2°C.

Fig. 4. 10.30—10.34 a.m. 10.9°—10.8°C.

Fig. 5. 10.35—10.40 a.m. 10.1°—9.6°C.

Fig. 6-8. Andere Gruppe.

Fig. 6. 8.25—8.35 a.m. 11.8°C.

Fig. 7. 9.30—9.40 a.m. 13.8°—15.2°C.

Fig. 8. 9.50—10.4 a.m. 10.7—10.5°C.



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